G.S. Saharan · Naresh Mehta



Sclerotinia Diseases of Crop Plants: Biology, Ecology and Disease Management



Springer

Sclerotinia Diseases of Crop Plants:
Biology, Ecology and Disease Management

G. S. Saharan • Naresh Mehta

Sclerotinia Diseases of Crop Plants: Biology, Ecology and Disease Management



Dr. G. S. Saharan
CCS Haryana Agricultural University
Hisar, Haryana, India

Dr. Naresh Mehta
CCS Haryana Agricultural University
Hisar, Haryana, India

ISBN 978-1-4020-8407-2 e-ISBN 978-1-4020-8408-9

Library of Congress Control Number: 2008924858

Printed on acid-free paper

9 8 7 6 5 4 3 2 1

springer.com

Foreword

The fungus *Sclerotinia* has always been a fancy and interesting subject of research both for the mycologists and pathologists. More than 250 species of the fungus have been reported in different host plants all over the world that cause heavy economic losses. It was a challenge to discover weak links in the disease cycle to manage *Sclerotinia* diseases of large number of crops. For researchers and students, it has been a matter of concern, how to access voluminous literature on *Sclerotinia* scattered in different journals, reviews, proceedings of symposia, workshops, books, abstracts etc. to get a comprehensive picture. With the publication of book on '*Sclerotinia*', it has now become quite clear that now only three species of *Sclerotinia* viz., *S. sclerotiorum*, *S. minor* and *S. trifoliorum* are valid. The authors have made an excellent attempt to compile all the available information on various aspects of the fungus *Sclerotinia*. The information generated so far has been presented in different chapters. After introducing the subject various aspects viz., the diseases, symptomatology, disease assessment, its distribution, economic importance, the pathogen, its taxonomy, nomenclature, reproduction, reproductive structures with fine details, variability, perpetuation, infection and pathogenesis, biochemical, molecular and physiological aspects of host-pathogen interaction, seed infection, disease cycle, epidemiology and forecasting, host resistance with sources of resistance, mechanism of resistance and other management strategies have been covered. The inclusion of numerous laboratory and field techniques is additional quality of the book for researchers, teachers and students. The chapters on *Sclerotinia* as myco-herbicide, phytotoxin, phytoalexins, hypo-virulence, resistance to fungicides, volatile compounds of *Sclerotinia*, sporigermin from sclerotia and *Sclerotinia* as health hazard problem will give a futuristic insight to the book. Outlining of future research priorities and disease management strategies speaks of the wisdom of the authors.

I congratulate Dr. G.S. Saharan, Ex Professor and Head, Department of Plant Pathology and Dr. Naresh Mehta, Professor of Plant Pathology, CCS Haryana Agricultural University, Hisar for their stupendous, incredible and splendor task of bringing comprehensive treatise on *Sclerotinia* which will propel fraternity of Agriculture to get bounty of knowledge at one edifice. I am sure this book will

be of immense help to the scientists, teachers, students, extension specialists and all those who are interested in protecting the plant health from *Sclerotinia* diseases.

October 2007 C.D. Mayee

Chairman
Agricultural Service Recruitment Board (ASRB)
Indian Council of Agricultural Research (ICAR)
Krishi Bhawan
New Delhi – 110 001

Preface

Sclerotinia is one of the most devastating and cosmopolitan plant pathogen. More than 60 names have been used to refer to diseases caused by this fungal pathogen. The fungus infects more than 500 species of plants worldwide including important field crops, fruit crops, ornamental plants, trees, shrubs and numerous weeds. Annual yield losses due to *Sclerotinia* diseases exceed over several hundred million dollars each year world over. Extensive crop damage, lack of high levels of host resistance and the general difficulty of managing diseases caused by *Sclerotinia* have been the impetus for sustainable research on this pathogen. Despite continued study by phytopathologists and mycologists, the taxonomic delimitation and relationship of the plant pathogenic species of *Sclerotinia* have never been resolved over the years, using traditional morphological and host preference characters.

The fungus *Sclerotinia* is belonging to phylum Ascomycota, class Discomycetes, order Helotiales, family Sclerotiniaceae has been redefined to include only those species that produce tuberoid sclerotia not incorporating host tissue within the sclerotial medulla developing an apothecial ectal excipulum composed of globose cells and not producing a disseminative conidial state. The taxonomy and nomenclature of 259 epithets previously referred to *Sclerotinia* have been reviewed with 21 placed in synonymy under the three accepted species and 25 included as imperfectly known. Two hundred and ten epithets have been excluded and either assigned or accepted to other genera. *S. homoeocarpa* causing "Dollar spot" in turf grasses now belonging to *Lanzia* sp. and *Moellerodiscus* sp. has been briefly covered as reference for readers. Now recently, with the increased information available on molecular biology, genetics, variability and epidemiology of these species and with reexamination in the light of micro-anatomical and cultural characters employed only three species, i.e., *Sclerotinia sclerotiorum*, *S. minor* and *S. trifoliorum* have been retained in this genus.

The present monograph on *Sclerotinia* deals with the aspects on taxonomy, nomenclature, geographical distribution, economic importance, host range, the diseases caused, symptomatology, disease assessment, reproduction, ultra structures, pathogenic variability, perpetuation, infection and pathogenesis, biochemical, molecular and physiological aspects of host pathogen interaction, seed infection, disease cycle, epidemiology and forecasting, host resistance and disease management strategies. In addition, laboratory and field techniques developed so far for *Sclerotinia* have been

included. Some newly emerging areas of *Sclerotinia* research which are likely to have a bearing on its management are *Sclerotinia* as myco-herbicide, phytotoxin, phytoalexin elicitors, hypovirulence, volatile compound imitator, sporigermin from sclerotia, resistance to fungicides and *Sclerotinia* diseases as health hazard problem have been discussed.

The subject matter is vividly illustrated with photographs (macroscopic, microscopic, electron micrographs, scanning electron micrographs), drawings, figures, histograms, graphs, tables and flow charts of techniques to make more interesting stimulating, effective and easy to understand by the readers. Each chapter is arranged in chronological order in the form of headings and sub-headings through numerical series to make the subject contiguous. Inclusion of most of the important references and websites will be helpful in original consultations by the *Sclerotinia* researchers, teachers and students.

We are sure that this comprehensive treatise on *Sclerotinia* will be of immense use to the scientists, teachers, students and all others in diagnosis and management of *Sclerotinia* diseases of crops worldwide.

G. S. Saharan
Naresh Mehta

Acknowledgements

Authors are indebted and highly grateful to the following persons/scientists/publishers/societies/journals/institutes/websites and all others whose valuable materials such as photographs (macroscopic, microscopic, electron micrographs, scanning electron micrographs), drawings, figures, histograms, graphs, tables, drawings and flow charts etc. have been used through reproduction in the present document. Authors are thankful to all the scientists/persons/societies/publishers/books/journals/institutes and websites etc. whose materials have been used in this document but have not been acknowledged inadvertently. The address of the author/source from where material adapted can be obtained from the reference which has been cited in the reference section of the book.

A Persons/Scientists

Abd-Elrazik, A.A.
Adams, P.B.
Agrios, G.N.
Ayers, W.A.
Boland, G.J.
Bolton, M.D.
Bullock, S.
Caesar, A.J.
Casale, W.L.
Casanova, S.
Cerkauskas, R.F.
Chandler, L.
Chen, C.
Dickman, M.B.
Dillard, H.R.
Dixon, G.R.
Doodson, J.K.
Dorrell, D.G.
Hart, I.P.
Hartill, W.F.T.
Hawthorne, B.T.
Hoes, J.A.
Holliday, P.
Huang, H.C.
Huang, R.C.
Hughes, K.J.D.
Imolehin, E.D.
Jain, J.P.
Jarvis, W.R.
Jeferies, P.
Jones, D.
Cummings, K.
Kapil, R.
Kapoor, A.S.
Kemp, W.
Kerr, E.W.

Ellis, M.B.
Fravel, D.R.
Gepp, V.
Godika, S.
Dr. Greg, E.
Grogan, R.G.
Gutteridge, C.S.
Hall, R.
Hao, J.J.
Martinez, A.
Mc Donald, M.R.
Mc Quilken, M.P.
McKenzie, D.L.
Melzer, M.S.
Millner, P.D.
Mordue, J.E.M.
Muralia, S.
Nelson, B.D.
Nelson, L.A.
Pathak, A.K.
Pearson, R.C.
Dr. Peter, L.
Rashid, K.Y.
Rowe, D.E.
Saito, I.
Shukla, A.K.
Silvera, E.
Singh, H.B.
Singh, P.
Singh, R.
Singh, S.
Smith, E.A.
Kerr, D.
Kohn, L.M.
Kokko, E.G.
Kora, C.
Lewis, J.A.
Lorbeer, J.W.
Lumsden, R.D.
Luth, P.
Spooner, B.M.
Steadman, J.R.
Subbarao, K.V.
Sugha, S.K.
Swanson, J.
Tariq, V.N.
Tewari, J.P.
Thomma, B.P.H.J.
Tricot, D.
Tripathi, N.N.
Tu, J.C.
Underhill, A.P.
Verma, P.R.
Waller, J.M.
Weiss, A.
Wharton, P.
Willetts, H.J.
Williams, M.A.J.
Wong, J.A.L.
Wu, B.M.
Young, C.S.

B Publishers/Societies/Journals/Institutes/Websites

Academic Press, USA
Blackwell Publishing Co., UK
British Mycological Society, UK
British Society for Plant Pathology, UK
CABI, Commonwealth Agriculture Bureau International, UK
Cambridge University Press, UK
Canadian Journal of Botany, Canada
Canadian Phytopathological Society, Canada

CCS Haryana Agricultural University, Hisar-India
Crop Science
Crop Science Society of America
Department of Scientific and Industrial Research, New Zealand
Elsevier Publishing Co., USA
Euphytica
Hokkaido Central Agricultural Experiment Station, Naganuma, Hokkaido, Japan
Indian Phytopathological Society, India
Indian Society of Mycology and Plant Pathology, India
International Society for Plant Pathology
Journal of General Microbiology
Journal of Phytopathology, Germany
Kluwer Publishers, USA
Michigan State University, USA
Micron
Molecular Microbiology
Molecular Plant Pathology
Mycological Research
Mycotaxon
North Carolina State University, USA
New Zealand Journal of Agricultural Research
Penn Sylvia State University, USA
Phytopathology
Plant Diseases
Scientific Publishers, USA
Springer SBM, The Netherlands
Taylor and Francis Group, FL
The American Phytopathological Society, USA
The National Research Council of Canada, Canada
The Netherlands Study Circles of Plant Breeding
The Royal Society of New Zealand, New Zealand
The Society for General Microbiology
University of Georgia, USA
USDA – Agricultural Research Service, USA
www.broad.mit.edu/annotation/fungi/sclerotinia/sclerotiorum
www.caes.uga.edu
www.ces.ncsu.edu
www.potatodiseases.org
www.sciencedirect.com
www.turfgrassmanagement.psu.edu
www.whitemoldresearch.com

(Authors)

Contents

List of Tables

List of Figures

List of Plates

Color Plates



Plate 5.2.7.1 White stem rot of rapeseed-mustard. (L–R) A: Initial growth at the lower portion of the stem; B: White mycelium growth acquires more areas; C, D: Infection on the stem caused drying of the branches; E: Drying of the crop visible in the field; F: Black hard sclerotia in side the pith of the stem (Photos A, B & F. Adapted from http: //www. whitemoldresearch.com. With permission)



Plate 5.2.8.1 *Sclerotinia* disease of soybean. A: Infected field showing dried plants; B: Infected stem at the basel portion of the stem (Adapted from http://www.whitemoldresearch.com. With permission)

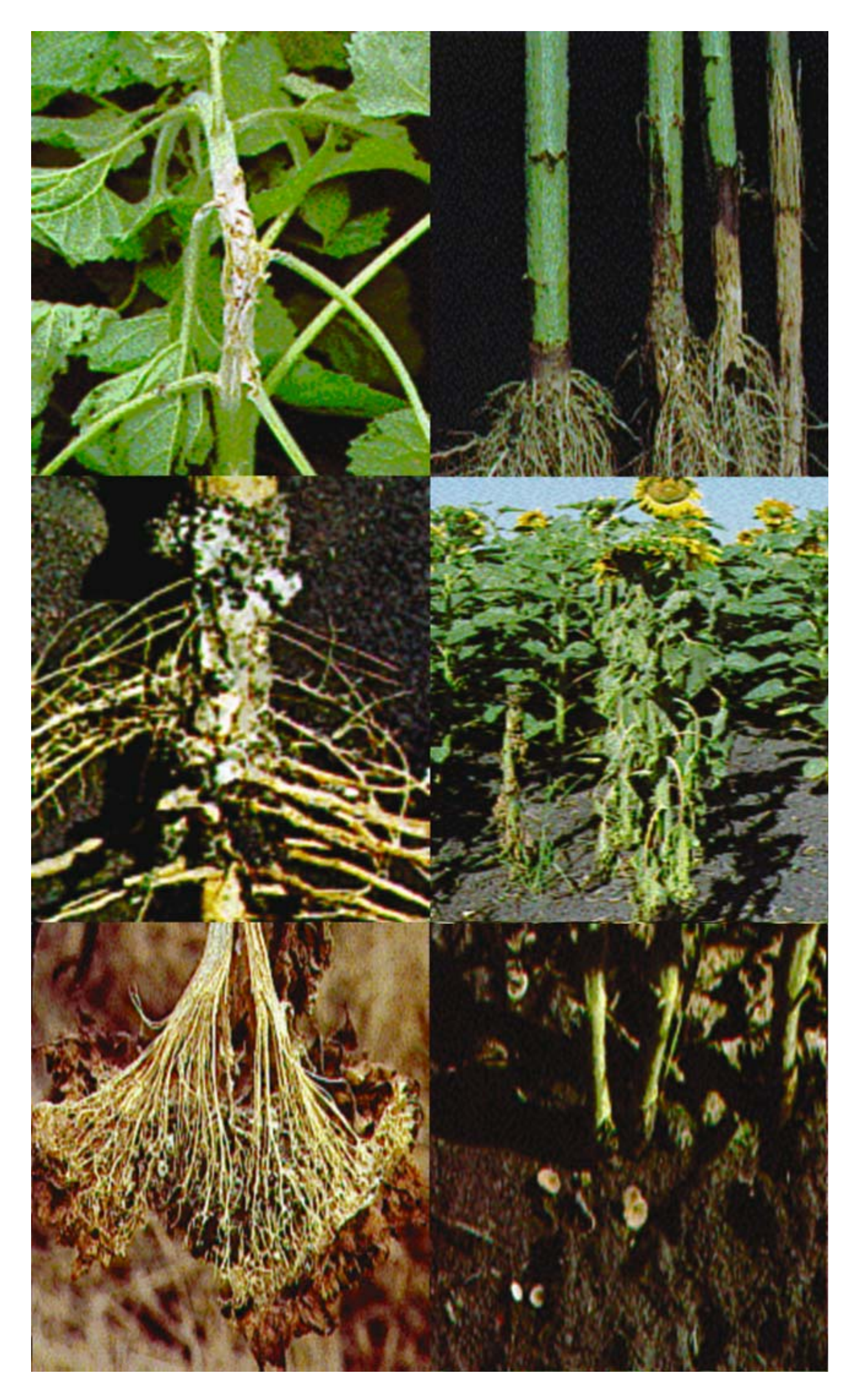


Plate 5.2.9.1 *Sclerotinia* disease of sunflower. (L–R) A: Mycelium growth at the middle of the stem; B: Basel canker formation at the base of the stem; C: White mould near the soil level; D: Sudden wilting of the plants in the field; E: Head rot due to *Sclerotinia* F: Apothecia formation on the soil (Adapted from http://www.whitemoldresearch.com. With permission)

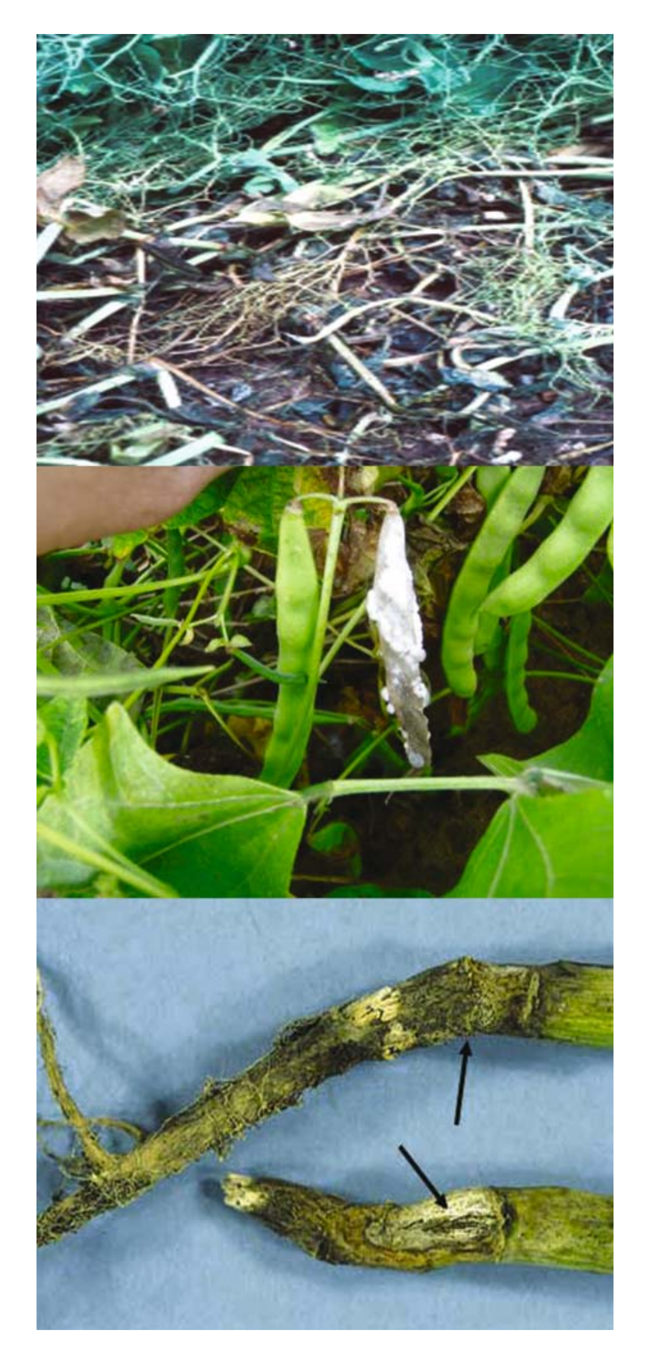


Plate 5.2.12.1 *Sclerotinia* disease of pea/beans. A: White mold infection on peas; B: White mold infection on pods; C: *Sclerotinia* infection at basel stem portion (Adapted from http: //www. white- moldresearch.com. With permission)



Plate 5.2.13.1 *Sclerotinia* disease of carrot. A. Leaves and petioles showing symptoms of *Sclerotinia*; B: Lesions advancing on carrot petioles; C: Collapsed leaves and petioles due to severe field infection; D: Sclerotia production on diseased leaves and soil surface; E: Secondary spread of foliar infection in the field; F: Mycelium of *S. sclerotiorum* erupting from the crown of a stored carrot originally infected in the field (Adapted from the publication of Kora et al., 2003. With permission)



Plate 5.2.17.1 *Sclerotinia* stem rot of potato. A, B: *Sclerotinia* infection at the base; C: *Sclerotinia* causing drying of the stem; D: Drying and breaking of the stem; E: Breaking and production of black sclerotia (Adapted from http://www.potatodiseases.org. With permission)



Plate 5.2.18.1 *Sclerotinia* disease of poppy; A: Basal rot of poppy; Abundant apothecial production under field conditions: B: Healthy (left) and infected (right) stem and mummified capsule of poppy; C: Flower buds heavily infected with pathogen showing white colony growth intermingled with sclerotia; D: Capsule of poppy showing sclerotia in side. E: Capsule of opium poppy showing infection of *Sclerotinia*, Black sclerotia on capsule; F: L.S. of infected (left) capsule showing fungal growth and sclerotia with healthy capsule (right) (Adapted from the publication of Singh and Singh, 2003. With permission)

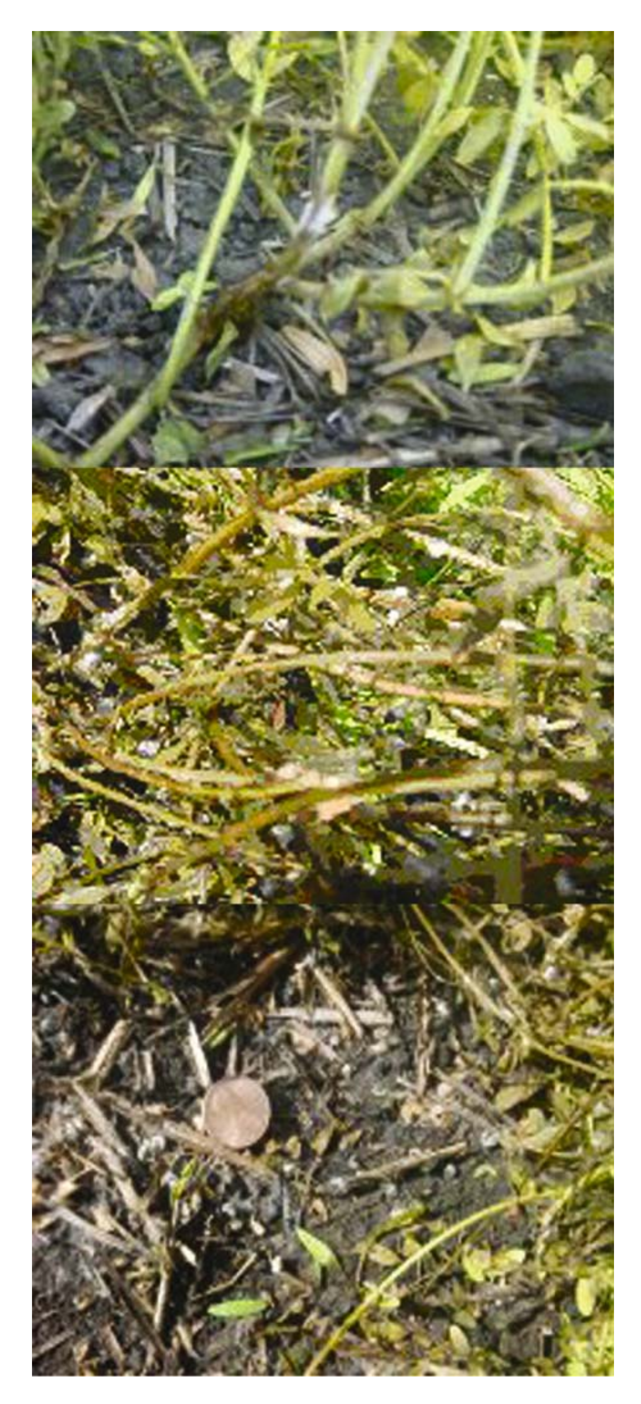


Plate 5.2.19.1 *Sclerotinia* rot in lentil. A: *Sclerotinia* disease infection on lentil stem; B: Severe infection at the base; C: Apothecia production at the soil level (Adapted from http:// www. whitemoldresearch.com. With permission)



Plate 5.2.24.1 *Sclerotinia* rot of chickpea. A: Drying of leaves at the initial infection; B: Severe infection cause drying of the stem; C: Mycelium and sclerotia formation at soil level; D: Sclerotia sticking to stem (Adapted from http:// www. whitemoldresearch.com. With permission)



Plate 5.2.25.1 Dollar spot of turfgrass. A: Dollar spot initiation on bent grass; B: Tan shaped lesions on the bent grass; C: Infected area on the leaf blade; D: Dense white mycelium on seedling turf; E: Large masses of hyphae/ mycelium on the lawns (Adapted from http://www.turf – grass management.psu.edu; http://www.caes.uga.edu; http://www.ces. ncsu.edu. With permission).

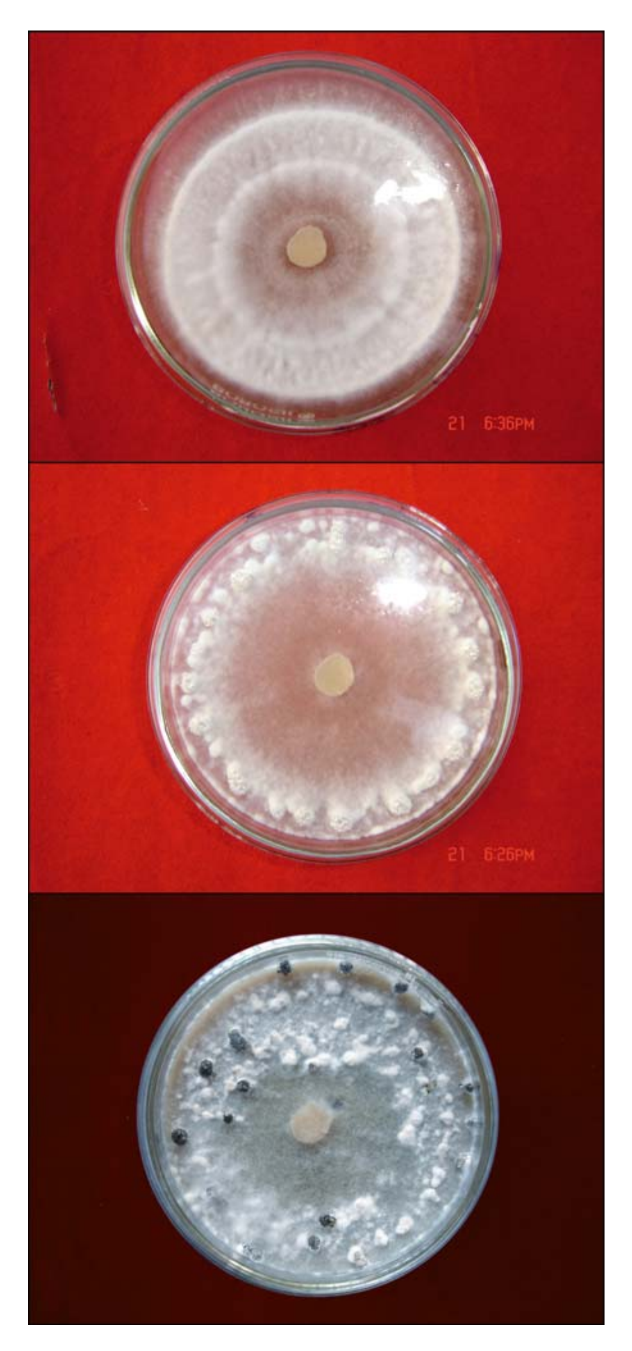


Plate 7.4.4.1 Growth pattern of *Sclerotinia sclerotiorum* (a) Mycelial growth; (b) Initaition of sclerotia formation; (c) Sclerotia formation at the outer periphery



Plate. 7.4.4.2 Apothecia formation in *Sclerotinia sclerotiorum*

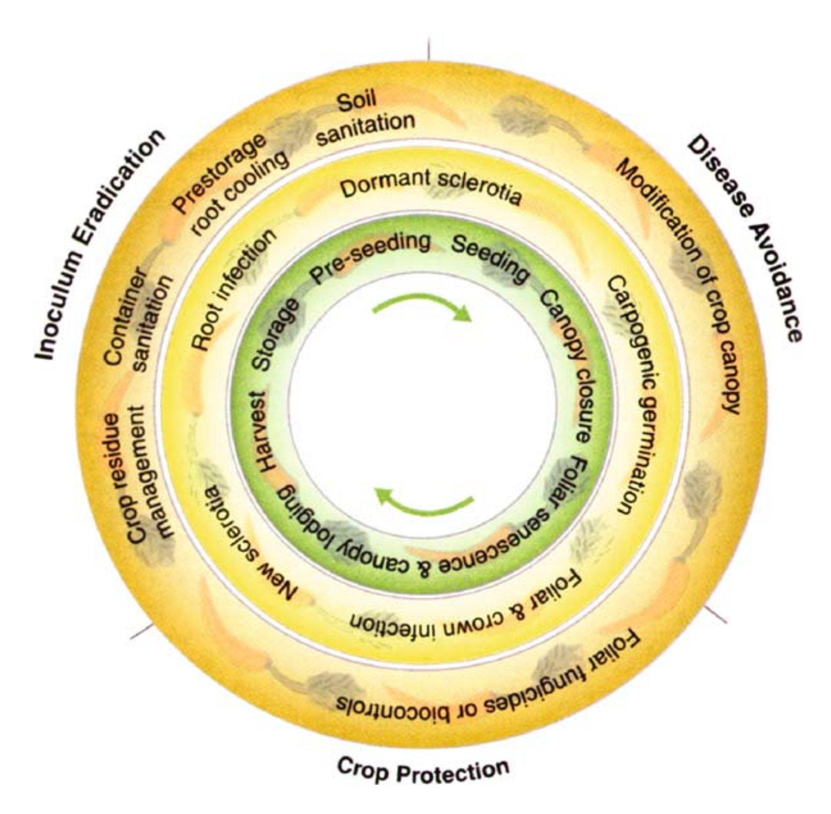


Fig. 19.11.1 An integrated model for managing *Sclerotinia* rot of carrot that incorporates three disease management principles and selected disease control strategies (outer circle), that target particular stages in the life cycle of *Sclerotinia sclerotiorum* (middle circle) or development of carrot crop (inner circle). Control strategies corresponding to respective stages are indicated by positional overlap (Adapted from the publication of Kora et al., 2003. With permission)

Biodata of G.S. Saharan



Dr. Govind Singh Saharan, Ex Professor and Head (Plant Pathology) retired from the active service during the year 2002. He did his B.Sc. Agriculture (1965), M.Sc. Agriculture (1967) from S.K.N. College of Agriculture, Jobner, University of Udaipur and Ph.D. from Himachal Pradesh University, Palampur (1977). He served as Lecturer (1967–1976) and Assistant Professor 1976–1980 at HPKVV, Palampur. Joined as Associate Professor (1980–1988), Professor (1988–2002) and Professor and Head (2002) of Plant Pathology at CCS, Haryana Agricultural University, Hisar.

Dr. Saharan has been visiting Professor to the University of Alberta, Edmonton, Canada (1991 and 1994), Agriculture, Canada, Saskatoon (1991, 1994, 1997) and Rothamsted, IACR, Harpenden, UK (1994 and 1997).

Dr. Saharan has more than 200 research publications in Journals of National and International repute. He has been editor of one book *Diseases of Oilseed Crops* and two other books. He is author of 25 review articles in books, monographs on White rust, Alternaria blight, Downy mildew diseases of rapeseed-mustard, and three bibliographies. He is on the panel of Experts of State Agricultural Universities, ICAR,

CSIR, UGC and DBT. He is one of the specialists to contribute in the release of Crop Protection Compendium, 2002, CAB International, UK. He has guided three M.Sc. and eight Ph.D. students who are well established scientists at different organizations in India and abroad.

Dr. Saharan has conducted research in diverse fields of Plant Pathology covering standardization of artificial inoculation technique, identification of sources of resistance, determination of pathogenic variability, genetics of host-parasite interaction, epidemiology and management of different diseases.

Dr. Saharan has been president of Indian Phytopathological Society (NZ) during 2001 and Editor-in-Chief of Indian Society of Mycology and Plant Pathology (1999–2002).

Dr. Saharan has been deeply associated with the organization of Global and Asian Congresses organized by the leading Phytopathological societies of India.

Biodata of Naresh Mehta



Dr. Naresh Kumar Mehta, Professor (Plant Pathology) did his B.Sc. Agriculture (Hons) from Haryana Agricultural University, Hisar in 1978 and attained first position in elective Plant Pathology. During his M.Sc. Agriculture (Plant Pathology), he was awarded Excel Industries Ltd. Bombay, India, research fellowship. He joined Haryana Agricultural University, Hisar as Assistant Scientist (Plant Pathology) in 1981 and completed Ph.D. degree as in-service candidate in 1993 in Plant Pathology with Dr. G.S. Saharan. He was awarded Ms. Manju Utereja Memorial Gold Medal for best Ph.D. thesis for the year 1993/94. He was also awarded Senior Research Fellowship (SRF) for Ph.D. programme by Council of Scientific and Industrial Research (CSIR), New Delhi.

Dr. Mehta has been admitted as Fellow of Indian Phytopathological Society, New Delhi, (FPSI), Indian Society of Plant Pathologist, Ludhiana (FSPP) and Indian Society of Mycology and Plant Pathology, Udaipur (FISMPP). He has been nominated as councilor (North Zone) of the society ISMPP for the year 2005/06. Dr. Mehta was elevated to the post of Associate Professor (Plant Pathology) in March 1994 and Professor (Plant Pathology) in 2002.

He has conducted research in diverse field of Plant Pathology covering pathogenic variability, genetics of host pathogen interaction, epidemiological studies, identification of resistant sources, biochemical/genetical basis for resistance, residual analysis of fungicides and disease management. He has been Co-Principal Investigator in the scheme: "Pathogenic variability and epidemiology of *Alternaria brassicae*" funded by ICAR, New Delhi, from December 1998 to May 2003. He has guided three M.Sc. (Plant Pathology) students.

He has published more than 75 research papers in the journals of National and International repute. He has one edited book (*Diseases of Oilseed Crops*), four review articles, 14 book chapters, six practical manuals, four lead lectures in the international and national conferences, 55 research papers presentations in the International/National conferences and about 35 popular articles to his credit.

Dr. Mehta has been a visiting scientist to University of Alberta, Edmonton, Canada in 1999 as a FAO fellow and presented a research paper in 8th International Congress of Plant Pathology at Christchurch, New Zealand, 2002. He has been invited to deliver a lecture in the 9th International Congress of Plant Pathology at Torino, Italy, 2008.

Chapter 1
Introduction

Sclerotinia is recognized as an important plant pathogen due to its worldwide distribution (Adams and Ayers, 1979; Lumsden, 1979; Purdy, 1979), its wide host range (Boland and Hall, 1994; Purdy, 1979; Schwartz et al., 1978) and the difficulties encountered in controlling the diseases it causes (Lumsden, 1979; Steadman, 1979; Walker, 1969). Although this fungus is most frequently found in regions tending to be cool and moist (Purdy, 1979). It is also reported to occur in some semi-arid regions where conditions would seem unfavourable for disease development (Blad et al., 1978; Purdy, 1979). Disease outbreaks in these drier areas occur during the summer months in irrigated fields since irrigation provides favourable microclimatic conditions for disease even though the macroclimate is unfavourable (Blad et al., 1978; Rotem and Palti, 1969), or the disease occurs during the cooler winter months (Purdy, 1979). Yield losses in susceptible crops vary and may be as high as 100 per cent (Purdy, 1979). *Sclerotinia* is generally considered to be a simple interest pathogen (Abawi and Grogan, 1979; Morrall and Dueck, 1982) utilizing ascospores as the primary inoculum. Although micro conidia are produced by this pathogen, their role, if any in its life cycle has not yet been shown (Walker, 1969; Willetts and Wong, 1980). Secondary spread of disease by plant to plant infection can occur by mycelial growth between stem bases or by contact between aerial parts of infected plants with neighboring healthy plants late in the growing season (Huang and Hoes, 1980; Morrall and Dueck, 1982).

Three species of *Sclerotinia* (*S. sclerotiorum*, *S. minor* and *S. trifoliorum*) at present are considered of much importance on the basis of wide distribution, host range and heavy yield losses caused in several crops of economic importance. Following the monographic revision of *Sclerotinia* by Kohn (1979a), *S. homoeocarpa* causing dollar spot of turf grasses may be more accurately classified within *Lanzia* sp. or *Moellerodiscus* sp. however, briefly it has been covered in symptomatology chapter. Two species with large sclerotia, *S. sclerotiorum* and *S. trifoliorum* are particularly similar as their sclerotia develop terminally by the repeated branching of primary hyphae. However, the former produces apothecia in the field during spring while the latter does so in the autumn and is confined to alfalfa and clover species, the former being plurivorous. *S. minor* has smaller sclerotia, which develop laterally by the repeated branching of short aerial hyphe, initials coalesce less frequently than in the first two species. The specific distinction of these taxa is further

G.S. Saharan, Naresh Mehta, *Sclerotinia Diseases of Crop Plants: Biology, Ecology and Disease Management*,

supported by the observation that hyphal contact between nonspecific isolates leads to unlimited growth over the contact zone, while between *S. sclerotiorum*, *S. trifoliorum* and *S. minor* incompatibility reactions occur.

The electrophoretic separation of proteins showed a clear distinction between these three species with little intra-specific variability. The electrophoretic patterns of soluble proteins and the enzymes aryl esterase, acid phosphatase, tetrazolium oxidase, glucose-6phosphate dehydrogenase and NADPH phosphate dehydrogenase also differed between these three species. Numerous Canadian isolates of *S. sclerotiorum* showed a considerable morphological, pathological and physiological variability, whereas isolates from different hosts in Britain showed a more continuous range of variation.

Sclerotia from infected plant tissues have a higher content of unsaturated lipids than those produced *in vitro*, low temperatures also favour the production of unsaturated lipids, sclerotia principally contain oleic but also smaller amounts of palmitic and linoleic acids.

Sclerotinia sclerotiorum is a polyphagous plant parasite with extremely voluminous phytopathological information spread over in the form of research papers, reviews, bulletins, leaflets, books and data base. Its distribution in the temperate zones is worldwide and the most important host plants are beans, potato, lettuce, sunflower, rape, safflower, soybean, peanuts, carrot, linseed, eggplant, cabbage, cauliflower, tomato, celery, chickpea, peas, lentil, buck wheat, capsicum, opium poppy and other vegetables in addition to large number of ornamental and medicinal plants. Mycelial isolations from soil have rarely been reported, but the sclerotia can be recovered quantitatively from infested soils by a combination of dry and wet-sieving and their viability tested on freshly cut carrot discs. Ascospores are the primary source of inoculum which infects above-ground parts of plants particularly through the petals, which can serve as an initial energy source, disintegrate within 72 h and facilitate the further spread of the fungus. Ascospores can survive particularly well for up to seven months at low humidity and germinate at osmotic potentials as low as −56 bars, but not at all at −91 bars. Sclerotia reach the soil mainly with decaying and ploughed-in plant material and can subsequently form daughter sclerotia, particularly in clay soil. *S. sclerotiorum* has been reported to be seed-borne in at least 27 host genera and the sclerotia can also be distributed with various seeds, particularly those of *Brassica* species. The sclerotia can be formed *in vitro* at osmotic potential between −1 and −64 bars. Survival times of five or up to ten years have been observed for sclerotia in the soil, particularly under dry conditions. Survival is adversely affected by high soil temperatures and moisture but is determined less by temperature and humidity than by microbial activities. Sclerotia buried in 5–20 cm depth of soil are still viable and produce apothecia after three to five years, whereas those nearer the soil surface disintegrate more rapidly. However, sclerotia do not form apothecia in soil at depths greater than 5 cm and the number produced from a sclerotium decreases with depth.

Apothecia are formed from sclerotia buried not deeper than 5 cm outdoors in soil or *in vitro* from well matured sclerotia kept at low temperatures (0 to +3°C) for two to several months and subsequently incubated on a moist, nutrient-poor substrate at

10–15°C under light. A constant temperature of 12–21°C during 60–90 days of sclerotium maturation has been found to give the shortest delay in germination, while temperatures fluctuating between 15–20°C results in 10 per cent abnormal fruit-bodies. Light is not only essential for disc differentiation but also strongly stimulates the development of stipe initials. Apothecia are produced only in moisture-saturated or near-saturated soils; the minimum water potential for this in heavy clay is −7.5 bars at 15°C. The activities of tyrosinase, lacoase, acid and alkaline phosphatases, esterases, malate, isocitrate, succinate and glucose-6-phosphate dehydrogenases are low in mature sclerotia but very much higher in developing apothecial initials, stipes and hypothecium. The sclerolial 1, 3-β D-glucanase is consumed during germination as 1, 3-β-D-glucanase activity increases. Cd^{2+} at 5×10^{-6}, Hg^{2+} at 5×10^{-5}, $C0^{2+}$ at 10^{-4}, Ni^{2+} at 5×10^{-5}, NaCl at 5×10^{-3}, Tris buffer at 2.5×10^{-3}, EDTA at 10^{-3} molar, and glucose and other sugars at 0.25 per cent concentrations selectively inhibit carpogenic sclerotium germination as do benomyl, dicloran and some other fungicides. A method of quantitatively collecting ascospores has been developed utilizing a Millipore filter funnel attached to a vacuum pump placed above sporulation apothecia; discharge can be induced by a short air flush once in three hrs. For optimal ascospore germination a high relative humidity is necessary; temperatures between 5°C and 10°C are suitable. In darkness, half as many sclerotia are formed as under 40–100 per cent of normal light intensity; numbers and sizes of sclerotia formed at different temperatures are inversely correlated, while growth rate and sclerotium formation are correlated with the diameter of the growth area; in large dishes, sclerotia form in concentric ring 0.5 cm apart. Sclerotium formation can be induced *in vitro* by acid staling compounds. Only actively growing hyphae from the margin of the colony give identical patterns of sclerotium production in subcultures. Sclerotia form *in vitro* in the osmotic potential range −1 to −64 bars, but not at −73 bars. Suitable C sources for sclerotium formation are raffinose, sucrose, maltose, lactose, D-mannose, D-glucose, D-fructose, D-galactose and L-arabinose; on the other hand, some of these sugars, and also a number of inorganic ions and organic compounds, can be released from growing or dried sclerotia. During sclerotium formation a high CO_2 evolution and high initial sugar (glucose) incorporation take place. The optimal temperature for mycelial growth lies in the range 15–25°C, depending on the isolate, while the optimal pH lies between 4 and 5.5. Growth is supported by araban, inulin and starch, in addition to the above-mentioned C sources, which also allow sclerotium formation. Mycelial growth is progressively stimulated as the osmotic potential decreases from −1 to −14 bars but it is reduced below the latter value; half the optimal growth rate is obtained in the range −37 to −47 bars. Growth over large distances in agar tubes is not limited by accumulating staling metabolites. During its decomposition of pectin, mainly endo- but also some exo-polygalacturonase, pectin methylesterase and pectin transeliminase have been detected. When grown on sterilized bean hypocotyls, adaptive cellulases with an optimum pH of 3.0 and a phospholipase A2 with an optimum pH of 4.0 are produced. Further enzymes investigated include an arbutine-splitting glucosidase, arabanase, a mycelium-bound trehalase, D-mannitol-1-phosphate, NAD oxidoreductase and D-mannitol,

NADP oxidoreductase, pentitol oxidoreductase (detected in cell-free extracts of mycelia and sclerotia grown on D-xylose, L-arabinose or D-ribose), oxalacetate acetylhydrolase, citrate synthetase, aconitate hydratase, NADP-specific isocitrate dehydrogenase, fumarate hydratase, malate dehydrogenase and acid phosphatase. The following compounds have been found to be suitable N sources, aspartic and glutamic acids, alanine, serine, glycine, tyrosine, peptone, sodium and potassium nitrate. Cysteine, cystine and methionine, lysine and urea, however, are not very suitable. In liquid culture, growth is depressed by deficiencies of phosphate, magnesium and trace elements and no significant growth occurs when there are nitrogen and potassium deficiencies. Oxalic, fumaric, succinic and glycolic acids are abundantly produced in later growth stages. Further metabolites reported include mannitol, acid phenols which can inhibit *Bacillus subtilis*, *Fusarium oxysporum* and *S. sclerotiorum* itself and the mycotoxins 8-methoxypsoralen and 4, 5, 8-trimethylpsoralen, both of which may cause dermatitis in man. This fungus is said, moreover, to have antibiotic properties against *Staphylococcus aureus*. Sclerin, the isocoumarin derivatives sclerotinin A and B, sclerone and isosclerone are responsible for growth-promoting effects at low concentrations and inhibition at higher concentrations in higher plants. The production of sclerin is correlated with sclerotium formation and pigmentation, and this compound apparently stimulates phenoloxidase and peroxidase activities. *S. sclerotiorum* is highly tolerant to Al^{3+} ions even at a pH of 3.7; consequently, phytotoxic levels of Al^{3+} can enhance its virulence on sunflower (Domsch et al., 1980).

Sclerotinia trifoliorum is primarily known as a pathogen of forage legumes such as alfalfa and clover, but attacks other uncultivated plants (Kohn, 1979b; Purdy, 1979; Scott and Evans, 1984; Willetts and Wong, 1980).

Sclerotinia minor has a host range of more than 100 plant species (Melzer et al., 1997) and additional hosts (after 1990) has been presented in Table 3.2.2.1 and is particularly severe in crops such as lettuce, sunflower, green beans, peanut and others.

Diseases like white mould, watery soft rot, cottony rot, white blight, stem rot, stem canker, stalk break, damping off, crown rot, wilt, blossom blight, drop and head rot are caused by three species of *Sclerotinia* (*S. sclerotiorum* (Lib.) de Bary, *S. minor* Jagger, *S. trifoliorum* Erikss.) in several crops grown all over the world. These diseases frequently cause serious and unpredictable yie1d losses in field and storage conditions of numerous crops. The disease incidence in different areas ranges from a trace to 100 per cent. The differences in incidence among fields are attributed to variations in environmental conditions, soil type, texture and drainage, cultural practices, inoculum density and disease management practices adopted in different localities (Purdy, 1979; Saharan, 1998). There are no simple ways of achieving total control of diseases caused by *Sclerotinia*. However, a well executed ecofriendly integrated control can manage the disease significantly and keep the yield loss to a minimum.

The symptoms caused by *Sclerotinia* vary somewhat with the host or host part affected and with the environmental conditions. The most obvious and typical early symptom of *Sclerotinia* diseases is the appearance on the infected plant of a

white fluffy mycelial growth in which soon afterwards develop large, compact resting bodies or sclerotia. Stems of infected succulent, herbaceous plants at first develop pale or dark brown lesions at their base. The lesions are often quickly covered by white cottony patches of fungal mycelium. In the early stages of lesion development in the stem, the foliage may show little sign of attack and infected plants are easily overlooked until the fungus grows completely through the stem and the stem rots. Then the foliage above the lesion wilts and dies more or less quickly. In some cases, the infection may begin on a leaf and then move into the stem through the leaf. The sclerotia of the fungus may be formed either internally in the pith of the stem, giving no outward signs of their presence there, or they may be formed on the outside of the stem where they are quite apparent. Succulent leaves and petioles suddenly collapse and die as the fungus infects the base of the stem and the lower leaves. Rapidly the fungus invades and spreads through the stem and the entire plant dies and collapses, each leaf dropping downwards until it rests on the one below. Fluffy, white mycelium and sclerotia appear on entire rotted plant parts.

The disease and its causal organisms have been subjects of intensive investigation. The etiology, biology and epidemiology of important *Sclerotinia* diseases have been studied extensively and summarized in several reviews, symposia and research publications (Abawi and Grogan, 1979; Adams and Ayers, 1979; Kohn, 1979a; Le Tourneau, 1979; Lumsden, 1979; Phillips, 1986a; Purdy, 1979; Rhodes et al., 1992). Nevertheless, the utility of reporter genes such as the green fluorescent protein (GPF) gene have already been exploited in *S. sclerotiorum* to gain more understanding on the biology of the fungus both *in vitro* and *in planta* (De Silva et al., 2005; Guimaraes and Stotz, 2004; Lorang et al., 2001). The pathogen over winter as mycelium in dead or living plant and as sclerotia on or within infected tissues or as sclerotia that have fallen on the ground. The sclerotia germinate and produce one to many slender stalks terminating at a small, 5–15 mm in diameter, disk of cup shaped apothecium in which asci and ascospores are produced. Primary infections are initiated chiefly by ascospores, which germinate with the help of free moisture and exogenous energy sources of fallen flower petals and necrotic tissues (Steadman, 1979). Other means of primary infection are associated with ascospore contaminated leaves in contact with moist soil and leaves coming in contact with sclerotia on the soil surface (Tu, 1989b). Secondary infections are achieved by natural contact of healthy plant parts with diseased ones. The epidemiology of *Sclerotinia* diseases is dependent on several factors viz., (a) soil inoculum, (b) soil type, (c) soil moisture, (d) rainfall, (e) soil and environmental temperature, (f) host susceptibility, (g) plant density and (h) cultural practices adopted. Diseases caused by *Sclerotinia* have traditionally been difficult to control. However, following management approaches which are ecofriendly and effective in control of *Sclerotinia* diseases of crop with best cost-benefit ratio have been advocated at different plate forums:

(a) **Field site selection**: The degree of field infestation by *Sclerotinia* species varies greatly. Such variation contributes in part to the difference in disease incidence in the fields ranging from 0 to 85 per cent. Farmers should know their fields.

Fields with a previous history of *Sclerotinia* diseases should be planted with resistant crops (Tu, 1997).

(b) **Cultural practices**: (i) *Field sanitation*: Any method that reduces the production, germination and/or survival of sclerotial inoculum significantly contributes to an effective management programme. Sclerotia sometimes are harvested along with seeds (sunflower, pea, bean, rapeseed etc.), therefore, the use of certified seed will reduce chances of introduction of the pathogen into clean fields. Redistribution of inoculum in infested crop straw, cull seeds or other residue into fields should be avoided. Burning of stubbles is highly effective control measure to destroy sclerotial inoculum, control of broad leaf weeds in all crops is useful, because many weeds are hosts of *Sclerotinia*.

(ii) *Tillage operations*: Most of the sclerotia near the top 2–3 cm of soil deteriorate within a year (Tu, 1997) and sclerotia buried deeper in the soil have a higher rate of survival. In the fields with a history of *Sclerotinia* diseases, a combination of proper crop rotation and no till operation reduces the risk of disease severity significantly.

(iii) *Mulching of the soil*: *Sclerotinia* stalk rot of cauliflower reduces significantly by mulching the soil with pine needles and sunflower inflorescence residues (Singh, 1983). Covering the plants with UV absorbing vinyl film (390 nm) reduces the total number of apothecia (Honda and Yunoki, 1980).

(iv) *Host nutrition*: Infection of sunflower plants is dependent on nutrition during growth. Placement of phosphorus during growth especially in mixture with humus reduces two to three times and considerably increases the yield (Polyakov, 1973). Micronutrient and slaked lime application are also reported to increase resistance of sunflower plants to the disease (Kochenkova and Polyakov, 1971).

(v) *Crop rotation*: At least three to five years crop rotations should separate sunflower and rapeseed from the preceding susceptible host crop (Morrall and Dueck, 1982; Zimmer and Hoes, 1978). In India, the cauliflower – rice – cauliflower or cauliflower – maize – cauliflower rotations are good to reduce disease severity (Singh, 1987).

(vi) *Date of planting*: The incidence and severity of *Sclerotinia* stem rot and wilt of gram is significantly less in November sown crop than on other dates (Singh and Singh, 1984a). November sown pea under Palampur (India) conditions result in no disease development (Singh, 1995). January sown sunflower crop gets less disease in comparison to the crop sown in October-November (Kolte and Tewari, 1977).

(vii) *Moisture regulation*: Flooding a field continuously for 23–45 days or cycles of alternate flooding and drying leads to destruction of sclerotia of *Sclerotinia* and reduces disease (Moore, 1949; Singh and Tripathi, 1996a). It has been shown that both apothecium production and disease severity are reduced by less frequent irrigation of bean (Blad et al., 1978).

(viii) *Soil solarization*: Solarization reduces the population of sclerotia of *Sclerotinia* in soil and reduces the ability of the surviving sclerotia to form apothecia. The greatest reductions occur in the top 5 cm layer of soil but significant effects are seen at 10 cm and 15 cm depths. During soil solarization sclerotia are completely killed at 45°C temperature after 3–4 h and at 35–40°C after 10–14 h (Chen

and Wu, 1990). Soil solarization with black polyethylene sheets is effective in reducing the number of apothecia of the pathogen (Wu, 1991). Solarization for 30 and 15 days affects recovery and viability of sclerotia in relation to depth of burial in the soil (Cartia and Aseri, 1994).

(ix) *Microclimatic modification*: An association between plant canopy development and *Sclerotinia* disease incidence and severity has been observed in various crops. Watering bean plants thoroughly until a continuous canopy forms, then reducing irrigation amount and frequency later in the season will result in less white mould and a stable yield (Steadman, 1979, 1983). Upright indeterminate and open bush type also results in reduced production of apothecia as compared with that under dense compact bush or vine types (Schwartz et al., 1978). In dry beans growing cvs. with a strong basal stem and narrow up right growth controls white mould disease (Huang and Kemp, 1989).

(x) *Host population and spacing*: Crop density is an important factor in determining disease incidence and severity. Planting fewer plants per row and increasing the row spacing should, therefore be an effective way to increase the rate of evaporation and thereby decrease the length of time where favourable conditions for disease development exist (Coyne et al., 1974; Steadman et al., 1973). In sunflower, the use of plant spacing of 36 cm or greater and plant population of 26–49 × 10^3 plants/ha would minimize yield loss due to *Sclerotinia* wilt (Hoes and Huang, 1976).

(c) **Seed treatment**: Seed treatments with fungicides like carbendazim, thiophanate methyl, benomyl, thiram, mancozeb and organomercurials have been reported to be effective for the control of *Sclerotinia* diseases. In pea, seed treatment with molybdenum and boron and a combined application of molybdenum with benomyl gives good control of *Sclerotinia* with increase in yield (Kuleshova, 1990).

(d) **Soil treatment**: Methyl bromide or formaldehyde has been used as effective pre-plant treatments for destroying sclerotia in the soil (Alabouvette and Louvet, 1973). Calcium cyanamide @ 500 kg/ha has been widely reported to prevent sclerotial germination and subsequent ascospore production (Kruger, 1973). Metham sodium @ 35 ml/M^2, methyl bromide @ 50 g/M^2 and soil solarization kills sclerotia in the top 10 cm soil and reduces apothecial production (Ben-Yephet, 1988). Long term use of calcium cyanamide increases soil fertility (Klasse, 1993). Thirty and 40 g/M^2 of methylbromide (MB) applied by using internally impermeable films to cover soil permits to achieve a very good control of *Sclerotinia* on lettuce in Italy (Gullino et al., 1996). The application of benomyl (0.5 kg a.i./ha), benomyl + mancozeb (0.5 + 1.6 kg a.i./ha), benomyl + iprodione (0.5 + 0.37 kg a.i./ha), iprodione (0.75 kg a.i./ha) and methyl thiophanate (1.5 kg a.i./ha) through irrigation water controls white mould of beans (Oliveira et al., 1995). Soil application of pesticides can be used in conjunction with bioagents of *Sclerotinia* in an integrated approach to disease control without toxic effect on biocontrol agents (Adams and Wong, 1991). Surface application of urea to soil @ 25–150 kg/ha is effective in controlling carpogenic germination of sclerotia. Ammonia released from decomposition of the urea appears to be the key toxic agent responsible for the inhibition of germination (Huang and Janzen, 1991).

(e) **Soil amendment**: Compost prepared from municipal sewage sludge is a valuable resource that can provide macronutrients and minor plant nutrients and improve the tilth and productivity of Agricultural soils. The per cent infection of lettuce seedlings by *S. minor* is reduced consistently 40–50 per cent by adding 10 per cent sewage sludge compost to the potting soil containing the pathogen (Lumsden et al., 1983). According to Asirifi et al. (1994) stable manure, fowl manure and lucerne hay are the best to reduce disease along with increase in yield. However, according to Huang et al. (1997), an amendment of field soil with either a formulation of fermented agricultural wastes, CF-5 or allyl alcohol at 150–400 ppm suppresses apothecial production of *S. sclerotiorum* and enhances the colonization of sclerotia of the pathogens by *Trichoderma* spp. Singh and Tripathi (1996b) has suggested use of 2 per cent neemgaurd along with usual nitrogen application in the soil to manage *Sclerotinia* rot of sunflower.

(f) **Biological control**: The time to implement biological control is in the resting (or sclerotium) stage of *S. sclerotiorum* during which the pathogen has little mobility, or at the germinating stage, during which the pathogen is most vulnerable to attack. Although, the number of biological control agents is very large (Table 19.9.1) but 15 fungi and 11 bacteria have shown their potentiality (Table 1.1) under field conditions to control *Sclerotinia* spp. and suppress diseases caused in different crops. *Coniothyrium minitans* and *Gliocladium virens* have shown practical potential for biological control of *S. sclerotiorum* (Budge et al., 1995). *C. minitans* applied to soil as a solid substrate inoculum can infect sclerotia of *S. sclerotiorum* year round and effectively reduce their number and viability in the soil (Budge et al., 1995; Gerlagh et al., 1994; Huang, 1977). Foliar application of spore suspension of *C. minitans* has been shown to reduce disease severity. The applications of spore suspension of *C. minitans* to crop residues occupied by *S. sclerotiorum* may reduce disease carry over and can be used in combination with soil incorporation treatments. Growing the fungus in the plant debris and reintroducing it into the soil can conceivably hasten the destruction of sclerotia in the field (Budge and Whipps, 1991; Trutmann et al., 1982; Whipps and Gerlagh, 1992). Considering development of resistance in biological agents like *Bacillus subtilis*, it should form part of integrated disease control (Li and Leifert, 1994).

Biological control of *Sclerotinia* diseases is pursued as an alternative disease control strategy. Under field conditions, sclerotia are attacked and degraded by a number of mycoparasites such as *Coniothyrium minitans* and *Sporidesmium sclerotivorum* have been proposed as biocontrol agents for this pathogen (Ayers and Adams, 1981a). *C. minitans* was discovered consistently from sclerotia in harvest samples, suggesting that this mycoparasite has potential in reducing survival of sclerotia in the field (Huang et al., 2000). However, there are some drawbacks to such agents. *S. sclerotivorum* is difficult to grow *in vitro*, limiting production of large scale quantities of the inoculum (del Rio et al., 2002). Nonetheless, to relieve the pressure on the environment posed by extensive use of chemicals to combat pathogens, biological control has recently been a hot topic for research with at least one commercial biocontrol product based on a *Sclerotinia* mycoparasite available to growers (Del Rio et al., 2002; Jones and Whipps, 2002; Reeleder, 2004).

Table 1.1 Potential biocontrol agents to control *Sclerotinia* species

Biocontrol agent	Effective against *Sclerotinia* species
Fungal species	
Coniothyrium minitans	*S. sclerotiorum*, *S. minor* & *S. trifoliorum*
Dictyosporium elegans	*S. sclerotiorum* & *S. minor*
Fusarium lateritium	*S. sclerotiorum*
Gliocladium catenulatum	*S. sclerotiorum*
Gliocladium roseum	*S. sclerotiorum*
Gliocladium virens	*S. sclerotiorum*
Penicillium citrinum	*S. sclerotiorum* & *S. minor*
Sporidesmium sclerotivorum	*S. sclerotiorum* & *S. minor*
Talaromyces flavus	*S. sclerotiorum*
Teratosperma oligocladium	*S. sclerotiorum*, *S. minor* & *S. trifoliorum*
Trichoderma harzianum	*S. sclerotiorum* & *S. minor*
Trichoderma koningii	*S. sclerotiorum*
Trichoderma pseudokoningii	*S. sclerotiorum*
Trichoderma viride	*S. sclerotiorum* & *S. minor*
Trichoderma roseum	*S. sclerotiorum*
Bacterial species	
Acinetobacter sp.	*S. sclerotiorum* & *S. minor*
Bacillus amyloliquefaciens	*S. sclerotiorum*
Bacillus cereus	*S. sclerotiorum*
Bacillus polymyxa	*S. sclerotiorum* & *S. minor*
Bacillus subtilis	*S. sclerotiorum* & *S. minor*
Pseudomonas spp.	*S. sclerotiorum*
Pseudomonas cepacia	*S. sclerotiorum* & *S. minor*
Pseudomonas chlororaphis	*S. sclerotiorum*
Pseudomonas fluorescens	*S. sclerotiorum*
Pseudomonas putida	*S. sclerotiorum* & *S. minor*
Staphylococcus spp.	*S. sclerotiorum*

(g) **Host resistance**: *Sclerotinia* has a wide host range without known strain specificity in pathogenicity. Many researchers formerly believed that resistance to *Sclerotinia* did not exist. Due to sporadic nature of disease outbreaks, especially for ascospore initiated disease as they are highly dependent on environmental conditions, screening for resistance under field conditions is often problematic. More recently, however, field resistance to this fungus has been observed in several crops (Table 18.5.1). Biotechnological approaches in enhancing disease resistance involve either exploitation of natural forms of resistance or genetic engineering, approaches such as the introduction of chitinases, glucanases and other antifungal proteins. The former approach may involve the introduction of novel resistance genes from wild species and the subsequent introgression of genes through the use of molecular markers or attempts to clone resistance genes. QTL for resistance to *Sclerotinia* have been reported in several important crops such as sunflower, common bean and oilseed rape (Arahana et al., 2001; Bert et al., 2004; Kolkman and Kelly, 2003; Miklas et al., 2003; Zhao and Meng, 2003a). Another strategy has been the introduction of a gene for oxalate oxidase in order to reduce susceptibility to *Sclerotinia* infection which relies upon the production of oxalic acid in the infection process.

Field resistance to *S. sclerotiorum* in some crops has correlated with laboratory resistance to oxalic acid (Kolkman and Kelly, 2000; Wegulo et al., 1998). A defense strategy against *S. sclerotiorum* in crop species is the use of transgenes that specifically degrade oxalic acid produced by *S. sclerotiorum*. The wheat germin gene coding for an oxalate oxidase catalyses oxidation of oxalic acid by molecular oxygen to CO_2 and H_2O_2 (Lane et al., 1993). H_2O_2 is also generated from the oxidative burst and has been implicated as an important factor in the plant HR (Levine et al., 1994; Wojtaszek, 1997). Further more H_2O_2 may be directly toxic to microbes (Peng and Kuc, 1992) or result in salicylic acid accumulation, an important signaling molecule in systemic acquired resistance (Gaffney et al., 1993; Leon et al., 1995). Therefore, transgenic oxalate oxidase in crop species might have indirect beneficial properties besides degradation of oxalic acid. Oxalate oxidase and other oxalic acid degrading enzymes have been incorporated into several important crops such as soybean, sunflower and peanut which have shown increased resistance to *Sclerotinia* spp. (Donaldson et al., 2001; Hu et al., 2003; Kesarwani et al., 2000; Livingstone et al., 2005). One of the disadvantages to this technology is the potential escape of transgenes into wild plants, possibly leading to more invasive wild species. A study on the environmental impact of oxalate oxidase transgenes escaping from cultivated sunflower into wild relatives revealed that oxalate oxidase does contribute to enhanced *S. sclerotiorum* resistance when backcrossed into wild flower. However, because the transgenes did not significantly affect seed production and reproductive output, the contribution of these transgenes did not give a fitness advantage to the new host plants, which suggests that oxalate oxidase may diffuse neutrally after a potential escape (Burke and Rieseberg, 2003).

(h) **Foliar application of fungicides**: As with the prevention of most diseases, chemicals to control those caused by *Sclerotinia* spp. must be applied before infection occurs. Since many *Sclerotinia* diseases are initiated by colonization of senescent plant organs, the fungicide must be applied to prevent colonization of these organs. Proper timing of spray and method of application have a great impact on results. Several registered fungicides, such as benomyl, chlorothalonil, thiophanate methyl, Iprodione and dicloran are effective and available but costly. However, fungicides have been used successfully on a commercial basis with soybean, dry bean, oilseed rape and some vegetables (Lamey et al., 2000; Budge and Whipps, 2001; del Rio et al., 2004; Twengstrom et al., 1998a). Although the development of resistance to fungicides is always a threat (Gossen et al., 2001). Disease production systems to aid in the efficiency of fungicide use have been implemented for some crops and are currently being developed for others (Clarkson et al., 2004; Davies et al., 1999; Gindrat et al., 2003; Turkington and Morrall, 1993).

(i) **Integrated disease management**: It is now very evident that several strategies must be combined into a single management programme in order to achieve maximum disease control. Crop rotation and weed control in non-host crops are essential for preventing the soil population of sclerotia from increasing, and reducing the crop density and planting crops with open canopy structures are effective ways to minimize disease development (Blad et al., 1978; Coyne et al., 1974; Kruger, 1980; Morrall and Dueck, 1982; Steadman, 1979; Williams and Stelfox, 1980b). Foliar sprays

such as Benomyl and soil fungicides such as calcium cyanamide are giving good results in the field on several crops.

The integrated effect of vermicompost, soil solarization, herbicide (EPTC), fungicide (procymidone), *Trichoderma harzianum* and *Bacillus subtilis* have been evaluated for the control of *S. sclerotiorum* by Pereira et al. (1996). Soil solarization through coverage of transparent polythene (0.1 mm) for 45 days is a good control strategy. EPTC treatment significantly increases the degree of control irrespective of the depth of the sclerotia in the soil.

Seed treatment, rotation between vegetable and cereal crops, fertilizers, rational close planting, pruning of old and infected leaves and 50 per cent carbendazole are used as integrated disease control measures for the control of rape *Sclerotinia* rot in Shanxi, China (Yu et al., 1995). For management of white mould of beans in Canada, Tu (1989c) suggested seed treatment with DCT (diazinon 6 per cent, captan 18 per cent, thiophanate methyl 4 per cent) to prevent the introduction of seed-borne *S. sclerotiorum* to disease free fields and the use of resistant cvs Ex Rico 23, Crestwood and Centralia.

Chapter 2
Geographical Distribution

Sclerotinia is one of the most devastating and cosmopolitan plant pathogens. More than 60 names have been used to refer to diseases caused by this fungal pathogen. The fungus infects over 500 species of plants worldwide. *S. sclerotiorum* has been reported from many countries located in all continents. The occurrence and distribution of *S. sclerotiorum*, *S. trifoliorum* and *S. minor* on various crops in the UK has been briefly reviewed by Davies et al. (1999). It is probable that the fungus occurs somewhere in almost every country (Purdy, 1979; Boland and Hall, 1994).

2.1 Distribution Map

2.1.1 Sclerotinia sclerotiorum

A distribution map is provided for *Sclerotinia sclerotiorum* (Lib.) de Bary Fungi: Ascomycota: Helotiales Hosts: Plurivorous. The pathogen has been reported from Europe, Albania, Austria, Belarus, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Malta, Moldova, Netherlands, Norway, Poland, Portugai, Romania, Central Russia, Russian Far East, Southern Russia, Serbia and Montenegro, Slovakia, Spain, Sweden, Switzerland, UK, Ukraine, Asia, Azerbaijan, China (Anhui, Fujian, Guangdong, Guangxi, Guizhou, Hainan, Hebei, Heilongjiang, Henan), Hong Kong (Hubei, Hunan, Jiangsu, Jiangxi, Jifin, Liaoning, Nei Menggu, Ningxia, Shaanxi, Shandong, Shanxi, Sichuan, Xinjiang, Xizhang, Yunnan, Zhejiang), Republic of Georgia, India (Arunachal Pradesh, Assam, Bihar, Delhi, Haryana, Himachal Pradesh, Jammu and Kashmir, Madhya Pradesh, Manipur, Meghalaya, Nagaland, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh, West Bengal), Iran, Israel, Japan (Hokkaido, Honshu, Kyushu, Ryukyu Archipelago, Shikoku), Jordan, Korea Republic, Lebanon, Nepal, Pakistan, Singapore, Syria, Taiwan, Tajikistan, Thailand, Turkey, Uzbekistan, Africa, Algeria, Congo, Egypt, Ethiopia, Kenya, Libya, Malawi, Mauritius, Morocco, Nigeria, South Africa (St. Helena),

G.S. Saharan, Naresh Mehta, *Sclerotinia Diseases of Crop Plants: Biology, Ecology and Disease Management*,

Tanzania, Zimbabwe, North America, Canada (Alberta, British Columbia, Manitoba, New Brunswick, Newfoundland, Nova Scotia, Ontario, Prince Edward Island, Quebec, Saskatchewan), Mexico, USA (Alabama, Alaska, Arizona, Arkansas, California, Colorado, Connecticut, Delaware, Florida, Georgia, Hawaii, Idaho, Illinois, Indiana, Owa, Kentucky, Louisiana, Maine, Maryland, Massachusetts, Michigan, Minnesota, Mississippi, Missouri, Montana, Nebraska, New Hampshire, New Jersey, New York, North Carolina, North Dakota, Ohio, Oklahoma, Oregon, Pennsylvania, Rhode Island, South Carolina, Texas, Vermont, Virginia, Washington, West Virginia, Wisconsin, Wyoming), Central America & Caribbean (bsermuaa), Costa Rica, El Salvador, Guatemala, Nicaragua, Panama, South America, Argentina, Bolivia, Brazil (Mato Grosso, Minas Gerais, Parana, Rio Grande Do Sul, Sao Paulo), Chile, Ecuador, Peru, Uruguay, Venezuela, Oceania, American Samoa, Australia (New South Wales, Queensland, South Australia, Tasmania, Victoria, Western Australia), Fiji, New Zealand, Samoa. (Distribution Map 971) as per Distribution Maps of Plant Diseases, 2005a, CABI, UK.

2.1.2 *Sclerotinia minor*

A distribution map is provided for *Sclerotinia minor* Jagger Fungi: Ascomycota: Helotiales Hosts: Lettuce (*Lactuca sativa*) and a range of other crop plants. It has been reported from Europe, Austria, Czech Republic, France, Germany, Hungary, Italy, Netherlands, Romania, Slovakia, Spain (Mainland Spain), UK, Yugoslavia (Fed.-Rep.), Asia, China, Hubei, Iran, Turkey, United Arab Emirates, Africa, Kenya, South Africa, North America, Canada (Ontario, Quebec), USA (Arizona, California, Maryland, New Jersey, New York, North Carolina, Oklahoma, Texas, Virginia), South America, Argentina, Brazil (Ceara, Sao Paulo, Colombia, Oceania), Australia (New South Wales, Queensland, Tasmania, Victoria, Western Australia, New Caledonia), New Zealand. (Distribution Map 889) as per Distribution Maps of Plant Diseases, 2003, CABI, UK.

2.1.3 *Sclerotinia trifoliorum*

A distribution map is provided for *Sclerotinia trifoliorum* Erikss. Hosts: Clover (*Trifolium*), lucerne (*Medicago sativa*), etc. It has been reported from Africa, Egypt, Asia, China, Israel, Japan, Korea, Australasia & Oceania, Australia, New Zealand, Europe, Austria, Belgium, Britain, Bulgaria, Czechoslovakia, Denmark, Finland, France, Germany, Greece, Hungary, Irish Republic, Italy, Netherlands, Norway, Poland, Romania, Sweden, Switzerland, USSR, North America, Canada, Mexico, USA, South America, Chile. (Distribution Map 274) as per Distribution Maps of Plant Diseases, 1982, CABI, UK

2.1.4 *Sclerotinia fructigena*

A distribution map is provided for *Sclerotinia fructigena* [*Monilinia fructigena*] Aderh. & Ruhl. Hosts: Apple (*Malus pumila*), pear (*Pyrus communis*), plum (*Prunus domesticus*), cherry (*P. cerasus* and *P. avium*). Information is given on the geographical distribution in Africa, Egypt, Morocco, Asia, Afghanistan, China (Honan, Yunnan), India (Punjab), Iran, Israel, Japan, Korea, Nepal, Turkey, Europe, Austria, Belgium, Britain & Northern Ireland (Channel Islands, Jersey), Bulgaria, Czechoslovakia, Cyprus, Denmark, Finland, France, Germany, Greece, Hungary, Irish Republic, Italy, Netherlands, Norway, Poland, Romania, Spain, Sweden, Switzerland, USSR (general), Yugoslavia, South America, Brazil, Chile, Uruguay. (Distribution Map 22) as per Distribution Maps of Plant Diseases, 1976a, CABI, UK.

2.1.5 *Sclerotinia laxa*

A distribution map is provided for *Sclerotinia laxa* [*Monilinia laxa*] Aderh. & Ruhl. (incl. f. sp. *mali* (Wormald) Harrison). Hosts: Stone fruit (*Prunus* spp.), apple (*Malus pumila*), pear (*Pyrus communis*). Information provides the geographical distribution of the fungus in Africa, Morocco, South Africa (Cape Province), Asia, Afghanistan, China (Chekiang, Kiangsu, Szechewan), Israel, Japan, Lebanon, Turkey, USSR, Central Asia, Abkhazia, Australasia & Oceania, Australia, New Zealand, Europe, Austria, Belgium, Britain & Northern Ireland, Bulgaria, Czechoslovakia, Denmark, Finland, France, Germany, Greece, Hungary, Irish, Republic Italy, Netherlands, Norway, Poland, Portugal, Romania, Spain, Sweden, Switzerland, USSR (Crimea, Ukraine, N. Caucasus), North America, Canada (British Columbia), USA (California, Oregon, Washington State, Wisconsin, Michigan, New York, Idaho), Central America & West Indies, Guatemala, South America, Argentina, Brazil, Chile, Uruguay. (Distribution Map 44) as per Distribution Maps of Plant Diseases, 1976b, CABI, UK.

2.1.6 *Sclerotinia fructicola*

A distribution map is provided for *Sclerotinia fructicola* [*Monilinia fructicola*] (Wint.) Rehm. Hosts: Stone fruit (*Prunus* spp.). Information is given on the geographical distribution of the pathogen in Asia, Japan, Australasia & Oceania, Australia, New Zealand, North America, Canada, USA, Central America & West Indies, Guatemala, Central America, South America, Argentina (Parana Delta), Bolivia, Brazil (Sao Paulo), Peru (Huanoco), Venezuela. (Distribution Map 50) as per Distribution Maps of Plant Diseases, 1976c, CABI, UK.

2.1.7 *Sclerotinia squamosa*

A distribution map is provided for *Sclerotinia squamosa* (Vienn. Bourg.) Dennis. Hosts: Onion (*Allium cepa*). The present information provides the geographical distribution of the pathogen in Africa, Mauritius, Asia, Hong Kong, Japan, Korea, Australasia & Oceania, Australia, New Zealand, Europe, Belgium, Britain, Bulgaria, France (Brittany), Italy, Netherlands, Poland, North America, Canada (Ontario, Quebec), USA, South America, Brazil (Rio Grande do Sul). (Distribution Map 164) as per Distribution Maps of Plant Diseases, 1977, CABI, UK.

2.1.8 *Sclerotinia narcissicola*

A distribution map is provided for *Sclerotinia narcissicola* Greg. Hosts: Narcissus. The pathogen has been reported from Australasia, Australia (Victoria), Tasmania, Europe, Channel Islands (Jersey; Guernsey), Denmark, Germany, Great Britain and Northern Ireland, Ireland (Republic), Netherlands, Norway, North America, Canada, United States. (Distribution Map 315) as per Distribution Maps of Plant Diseases, 1968a, CABI, UK.

2.1.9 *Sclerotinia borealis*

A distribution map is provided for *Sclerotinia borealis* Bubak & Vleugel. Hosts: Cereals and grasses. Information is given on the geographical distribution in Asia, Japan, USSR (Soviet Far East, Sverdlovsk), Europe, Finland, Norway, Sweden, USSR (Gorki, Kirov, Leningrad, Ukraine), North America, Canada (B.C., Yukon), USA (Alaska, Washington State). [Distribution Map 446] as per Distribution Maps of Plant Diseases, 1968b, CABI, UK.

2.1.10 *Sclerotinia fuckeliana*

A description is provided for *Sclerotinia fuckeliana*. Hosts: Occurs both as a parasite and a saprophyte on a very wide range of host plants. Disease: Causes ‘grey mould’ or ‘botrytis disease’, a blight or rot of immature, fleshy or senescent tissues. Lesions develop as tan or brown water soaked areas, which may become greyish on drying out. The profuse grey brown sporulation of the fungus on old diseased tissue is characteristic. Rotting of perishable plant produce at harvest or in store causes large losses; can be particularly severe on soft fruit such as strawberries and grapes and vegetables such as cabbage, lettuce etc. Damping-off and basal leaf and stem rot

result in severe damage to lettuce and flax. Blights of buds, blossom, leaves and stems may also occur on a wide range of hosts and the fungus has been implicated in die-back and canker formation on woody plants. Information has been provided for its geographical distribution that it is World wide, but most prevalent as a disease in humid temperate or sub-tropical areas. Transmission: Conidia are air-borne, but may be carried on the surface of rain splash droplets. Diseased plant parts, on which sporulation is profuse in wet weather, are important sources of inoculum in disease epidemics. The fungus over winters as sclerotia or as mycelium in old plant debris and may be seed borne as spores or mycelium on host. [Distribution Map 431] as per Distribution Maps of Plant Diseases, 1998, CABI, UK (Ellis and Waller, 1998).

Chapter 3
History and Host Range

3.1 History

Although the importance of *Sclerotinia* as a plant pathogen has long been known but detailed historical account of *Sclerotinia* was given by Purdy (1979). In 1837, Liebert described *Peziza sclerotiorum*. Fuckel (1870) erected and described the genus *Sclerotinia*. He chose to honour Liebert (1837) by renaming *Peziza sclerotiorum* with a newly coined binomial, *Sclerotinia libertiana*. According to Wakefield (1924), Fuckel apparently disliked the combination of *S. sclerotiorum* and elected to establish the new one. *S. libertiana* Fuckel was in use until Wakefield (1924) showed it to be inconsistent with the International Rules of Botanical Nomenclature and cited G. E. Massee as the proper authority for *Sclerotinia sclerotiorum* (Lib.) Massee because he has used that binomial up to 1895, but de Bary used it in his 1884 contribution. Thus, the proper name and authority for this pathogen seems to be *Sclerotinia sclerotiorum* (Lib.) de Bary. Eriksson (1880) described the pathogen of clover stem rot as *Sclerotinia trifoliorum* Erikss. Then Wolf and Cromwell (1919) suggested that clover stem rot may have present near Berberbeck in Hesse, Germany in 1857. It was also mentioned that the disease name may be synonymous with the name clover sickness, a disease known in England in the early 1800s and on which personnel at Rothamsted Experiment Station began work in 1849. However, the disease apparently was attributed to *S. trifoliorum* in 1897. According to Wolf and Cromwell (1919), the first report of *S. trifoliorum* in the USA was published in Delaware in 1890. Jagger (1913) described the small sclerotial type from lettuce, celery and other crops in several locations in New York and from lettuce in Sanford, Florida as *S. minor*. Valleau et al. (1933) suggested that *S. minor* and *S. trifoliorum* are identical with *S. minor* occurring on host (lettuce) not commonly recognized as a host for *S. trifoliorum*, thus, associated host and size of sclerotia were used as the basis for speciation of *Sclerotinia* isolates. Additional species were named as *S. intermedia* Ramsy, *S. serica* Keay, *S. trifoliorum* Erikss. var. *fabae* Keay and *S. sativa* Drayton and Groves. Dennis (1956) included *S. sclerotiorum*, *S. trifoliorum* (also the variety *fabae*), *S. minor*, *S. serica*, and *S. tuberosa* but did not mention *S. intermedia* or *S. sativa*, it suggests that these latter two as valid species were not recognized or that

G.S. Saharan, Naresh Mehta, *Sclerotinia Diseases of Crop Plants: Biology, Ecology and Disease Management*,

these species occur only in the new world. It appears that others share concepts or parts of concepts with Dennis, because *S. intermedia*, *S. sativa*, and *S. serica*, along with *S. trifoliorum* var. *fabae* seem to have disappeared from the literature as if they were the "Putdown men" of *Sclerotinia* spp.

Certain diseases caused by *S. sclerotiorum* may serve as indicators of the history (Purdy, 1979). Lettuce drop is a classical example where Smith (1900) demonstrated beyond question that *S. sclerotiorum* (used *S. libertiana*) caused "drop" and that *S. sclerotiorum* and *Botrytis cinerea* are different fungi. These two fungi produced similar disease of lettuce, but the true drop caused by *S. sclerotiorum*, was more common on glasshouse lettuce in Massachusetts in the late 1890s than was the disease caused by *B. cinerea*. Stevens and Hall (1911) reported that lettuce drop occurred in Massachusetts in 1890, Florida in 1896, North Carolina in 1897 and Wisconsin in 1904. In contrast, Burger (1913) described the occurrence of lettuce drop in the vicinity of Gainesville in 1896 and in North Carolina in 1897. But it was not until 1900 that this classic disease was attributed to *S. libertiana* (*S. sclerotiorum*) in Massachusetts. Jagger (1913) stated that the fungus Smith (1900) had described was the same one he had observed associated with lettuce in several areas of New York and suggested that this fungus was an "undescribed" species of *Sclerotinia*. Thus, Jagger (1920) described the small-sclerotia type as *Sclerotinia minor*. Held and Haenseler (1953) suggested that severe attacks of lettuce drop in the field planted for the first time following clover or lucerne (alfalfa) may be caused by *S. trifoliorum*. Gilbert and Bennett (1917) as well as Wolf and Cromwell (1919) stated that Rhem in 1872 designated the pathogen of clover stem rot as *Peziza ciborioides* Fries, a name considered to be untenable by Eriksson (1880) who described the pathogen as *S. trifoliorum*. The first report of clover stem rot in the USA was in 1890 by Wolf and Cromwell (1919). According to Jones (1923), stalk rot of sunflower is caused by *S. libertiana* (*S. sclerotiorum*). Although, *Sclerotinia* blight of peanut had been reported in China in 1935 and in Argentina in 1950 but in the USA, it was reported in 1974 (Beute et al., 1975). Smith (1929) described cottony rot of lemons. Later Smith (1929) described the life history of *S. sclerotiorum* in relation to green fruit rot disease of apricot. Limb blight of fig was reported by Taubenhaus and Ezekiel (1929). Dickson (1930) reported wilt of greenhouse tomatoes and confirmed the pathogen to be *S. libertiana* (*S. sclerotiorum*). White mould of beans was dealt in detail by Harter and Zaumeyer (1944).

The Sclerotiniaceae is a family of fungi in the order Helotiales in the phylum Ascomycota. The Sclerotiniaceae includes species producing inoperculate asci from brownish stipitate apothecia that arise from a sclerotial stroma within or associated with a host plant (Whetzel, 1945). The development of a sclerotial stroma, a melanized hyphal aggregate is the common character of all members of the Sclerotiniaceae. Although such teleomorphic features are strongly conserved in the Sclerotiniaceae, there is large diversity in the anamorphic state, which has been the impetus for a separation of genera within the family (e.g. *Monilinia* for species with *Monilia* anamorphs, *Botryotinia* for species with *Botrytis* anamorph, etc.) which was supported by Kohn (1979b). Additional taxonomic criteria used since Whetzel's (1945) delimitation of the Sclerotiniaceae include characteristics of sterile tissues of apothecia and sclerotia (Kohn, 1979a, b; Korf and Dumont, 1972), sclerotial ontogeny

(Willetts and Wong, 1980), histochemistry and ultrastructure of sclerotia (Backhouse and Willetts, 1984), biochemical characteristics (Carbone and Kohn, 1993) and rRNA gene sequences (Holst-Jensen et al., 1997a, b). Currently, 33 genera have been recognized (Willetts, 1997).

The distribution of species within the genus has been revisited several times. Systems of species separation within *Sclerotinia* have been focused on size of sclerotia (Jagger, 1920), host association (Kreitlow, 1949), ascus and ascospore size (Ramsey, 1924), or nuclear and mitochondrial RFLP analyses (Kohn et al., 1988). As universally accepted, three valid species remain in *Sclerotinia* sensu strict to: *S. minor* Jagger, *S. trifoliorum* Erikss and *S. sclerotiorum* (Lib.) de Bary (Kohn et al., 1988). Further it was reported that *S. asari* Wu and Wang (Wu and Wang, 1983) and *S. nivalis* Saito (Li et al., 2000; Saito, 1997) were distinct members of *Sclerotinia* based on DNA analysis. Another species *S. homoeocarpa* F. T. Bannett is not considered to be valid but has not been formally reclassified (Kohn, 1979b; Rossman et al., 1987).

Sclerotinia sclerotiorum was first described in 1837 as *Peziza sclerotiorum* (Liebert, 1837). This binomial stood until the species was transformed to the new genus *Sclerotinia* (Fuckel, 1870) and renamed *Sclerotinia libertiana* Fuckel in honour of Libert (Purdy, 1979) with *Peziza sclerotiorum* Lib. and *S. sclerotii* Fuckel cited as synonyms (Wakefield, 1924). Mycologists and plant pathologist accepted and used *S. libertiana* until Wakefield (1924) showed it to conflict with the International Code of Botanical Nomenclature as a species that is transferred from one genus to another must retain the original specific name, unless the resulting combination is already occupied. In this case, *Sclerotinia sclerotiorum* was not already taken. However, Wakefield (1924) incorrectly reported that the combination of *S. sclerotiorum* was first used by G. E. Massee in 1895, resulting in the citation *S. sclerotiorum* (Lib.) Massee. Purdy (1979) observed that de Bary used the name in 1884 and therefore, the proper name and authority for the fungus should be *Sclerotinia sclerotiorum* (Lib.) de Bary.

In addition to the confusion regarding the correct name for the fungus, there has also been uncertainty regarding the correct type specimen (Korf and Dumont, 1972). To resolve this issue, the type species for the genus *Sclerotinia sclerotiorum* (Lib.) de Bary, was proposed for conservation in 1973 by Buchwald and Neergaard (Kohn, 1979b) and accepted as a conserved name in 1981.

3.2 Host Range

3.2.1 Sclerotinia sclerotiorum (Lib.) de Bary

Sclerotinia sclerotiorum appears to be among the most nonspecific, omnivorous and successful plant pathogen. The broad host range of this fungus is important to the control of disease in agricultural crops because it restricts the number of non host crops that can be included in crop rotations designed to reduce the concentration of sclerotia in infected soils. The extensive host range of this pathogen restricts its use as a mycoherbicide because of the potential for dissemination of inoculum to non

target hosts. Determining the risk of disease in non target plants is difficult because of the lack of a readily available and comprehensive host index of *S. sclerotiorum*. Records of susceptible hosts of this pathogen are scattered throughout the unpublished and published scientific literature. Partyka and Mai (1962) indicated that 172 species from 118 genera in 37 plant families are known to be susceptible hosts. Farr et al. (1989) listed 148 genera of plants that are susceptible to *S. sclerotiorum*. Schwartz (1977) reported a host range of 374 plant species from 237 genera in 65 families. Purdy (1979) referred to a compilation by P. B. Adams that included 361 species from 225 genera in 64 families. The most recent host index for *S. sclerotiorum* (Table 3.2.1.1) prepared by Boland and Hall (1994) contains 42 subspecies or varieties, 408 species, 278 genera, and 75 families of plants. There is considerable variation in the clarity of information provided in reports of new hosts of *S. sclerotiorum*. Changes in nomenclature of the fungus and the hosts represented one source of variability. A compilation of these records into a host index of *S. sclerotiorum* would facilitate analysis of the risk of using biological control products containing this pathogen. The critical determination of the host range of this pathogen is essential to identify potential source of disease resistance, consolidate our knowledge of the host specificity of this pathogen and fungal evolution. Except for one species of the Pteridophyta, all hosts of *S. sclerotiorum* occur in the classes Gymnospermae and Angiospermae of the Division Spermatophyta. Most hosts are herbaceous plants from the subclass Dicotyledoneae of the Angiospermae but several hosts also occur in the subclass Monocotyledonae. The additions in the host range in *Sclerotinia sclerotiorum* since 1990 are presented in the Table 3.2.1.2.

3.2.2 Sclerotinia minor Jagger

An index of plants reported to be susceptible to *S. minor* has been compiled from the scientific literature by Melzer et al. (1997). The index contains 21 families, 66 genera and 94 species. All hosts of *S. minor* occur within the class Angiospermae of the plant division Spermatophyta. Most hosts are from the subclass Dicotyledonae although two are from the subclass Monocotyledonae. It is hoped that this index will be useful for planning crop rotations and identifying weeds that act as reservoir hosts, identifying potential sources of disease resistance, facilitating a risk analysis for using *S. minor* as a mycoherbicide and for consolidating knowledge of the host-specificity of this pathogen. Some additions as susceptible host plants to *S. minor* are presented in Table 3.2.2.1.

3.2.3 Sclerotinia trifoliorum Erikss

Sclerotinia trifoliorum appears to be cosmopolitan species with a host range limited to forage legumes. The fungus *S. trifoliorum* attacks mainly to forage legumes and

Table 3.2.1.1 Host range of *Sclerotinia sclerotiorum* (Adapted from publication of Boland and Hall, 1994. With permission)

Latin binomial	Common name
POACEAE (Grass family)	
Avena sp.	Oats
Digitaria sanguinalis (L.) Scop. var. *ciliaris* (Retz.) Parl.	Large crab grass
Hardeum vulgare L.	Barley
Pennisetum americanum Schum.	Pearl millet
Secale cereale L.	Rye
Setaria viridis (L.) Beauv.	Green foxtail
Sorghum bicolor (L.) Moench	Broomcorn
Sorghum vulgare Pers.	Sorghum
Triticum aestivum L.	Wheat
Zea sp.	Maize
FABACEAE (Pulse family)	
Apios americana Medic.	Groundnut
Arachis hypogaea L.	Peanut
Astragalus sinicus	Milk vetch
Cicer arietinum L.	Chick pea
Caronilla varia L.	Crown vetch
Crotalaria juncea L.	Sun hemp
Desmodium triflorum L. D.C.	Undetermined
Dolichos biflorus L.	Horse gram
Dolichos lablab L.	Egyptian bean
Glycine max (L.) Merrill	Soybean
Lathyrus esculenta Moench (as *L. culinaris* Medic.)	Lentil
Lathyrus odoratus L.	Sweet pea
Lathyrus sativus L.	Grass pea
Lens culinaris Medic.	Lentil
Lotus corniculantus L.	Bird's-foot trefoil
Lotus sp.	Undetermined
Lupinus angustifolius L.	European blue lupine
Lupinus nootkatensis Donn. (as *L. regalis* Berg.)	Lupine
Lupinus perennis L.	Sundial lupine
Lupinus polyphyllus Lindl.	Washington lupine
Lupinus sp.	Lupine
Medicago hispida Gaertn.	Bur clover
Medicago lupulina L.	Black medick
Medicago sativa L.	Alfalfa
Melilotus alba Desr.	White sweet clover
Melilotus indica (L.) All.	Sweet clover
Melilotus officinalis (L.) Pall.	Yellow sweet clover
Melilotus sp.	Sweet clover
Onobrychis vicifolia Scop.	Sainfoin
Phaseolus cocccineus L.	Scarlet runner bean
Phaseolus limensis Macfady	Lima bean
Phaseolus lunatus L.	Civet bean
Phaseolus radiatus L.	Green bean
Phaseolus vulgaris L.	Kidney bean

(continued)

Table 3.2.1.1 (continued)

Latin binomial	Common name
Pisum sativum L.	Pea
Pisum sativum L. var. *arvense* (L.) Poir.	Field pea
Stylosanthes hamata (L.) Taub.	Caribbean stylo
Stylosanthes humilis Kunth.	Townsville stylo
Stylosanthes guianensis (Aubl.) Sw.	Brazilian stylo
Trifolium alexandrinum L.	Egyptian clover
Trifolium hybridum L.	Alsike clover
Trifolium incarnatum L.	Crimson clover
Trifolium procumbens L. (as *T. dubium* Sibth)	Least hop clover
Trifolium pratense L.	Red clover
Trifolium pratense L. var. *praecox*	Undetermined
Trifolium pratense L. *var. seratinum*	Undetermined
Trifolium repens L.	White clover
Trifolium sp.	Clover
Trifolium subterraneum L.	Subterranean clover
Trifolium wormskjoldii Lehm. (as *T. fimbriatum* Lindl).	Sierra clover
Trigonella foenum-graecum L.	Fenugreck
Trigonella sp.	Fenugreck
Vicia faba L.	Broad bean
Vicia sativa L.	Spring vetch
Vicia sp.	Vetch
Vicia villosa Roth.	Hairy vetch
Vigna angularis (Willd.) Ohwi & Ohashi (as *Phaseolus angularis* (Willd.) Wright)	Azuki bean
Vigna mungo (L.) Hepper (as *Phaseolus mungo* L.)	Black gram
Vigna radiata (L.) Wilcz. (as *Phaseolus aureas* Roxb.)	Mung bean
Vigna unguiculata (L.) Walp. (as *V. sesquipedalis* (L.) Frow.	Yard-long bean
Vigna unguiculata (L.) Walp. sub sp. *sesquipedalis* (as *V. sinensis* (Torner) Savi.)	Cowpea
BRASSICACEAE (Mustard family)	
Arabidopsis thaliana (L.) Heynh.	Mouse-ear cress
Armoracia rusticana Gaertn., Mey. & Scherb.	Horseradish
Barbarea vulgaris R Br.	Yellow rocket
Brassica campestris L.	Bird rape
Brassica campestris L. var. *napobrassica* (L.) D.C.	Rutabaga (Swede)
Brassica chinensis L.	Pak-choi
Brassica hirta Moench.	White mustard
Brassica juncea (L.) Coss.	Leaf mustard
Brassica juncea (L.) Coss. var. *crispifolia* Bailey	Curled mustard
Brassica kaber (D.C.) Wheeler	Charlock
Brassica napus L.	Rape
Brassica nigra (L.) Koch	Black mustard

(continued)

Table 3.2.1.1 (continued)

Latin binomial	Common name
Brassica oleracea L. var. *acephala* D.C.	Kale
Brassica oleracea L. var. *botrytis* L.	Broccoli
Brassica oleracea L. var. *botrytis* L.	Cauliflower
Brassica oleracea L. var. *capitata* L.	Cabbage
Brassica oleracea L. var. *caulorapa* D.C.	Knolkhol
Brassica oleracea L. var. *gemmifera* Zenk.	Brussels sprouts
Brassica oleracea L. var. *gongylodes* L.	Kohlrabi
Brassica oleracea L. var. *ramosa* Alef.	Undetermined
Brassica oleracea L. var. *viridis* L.	Kale
Brassica pekinensis (Lour.) Rupr.	Chinese cabbage
Brassica rapa L.	Turnip
Brassica rugosa Lai	Undetermined
Capsella bursa-pastoris (1.) Medic.	Shepherd's purse
Cardamine heterophylla (Forst.) Schutz	Bittercress
Cheiranthus chieri L.	Wallflower
Crambe abyssinica Hochst. ex R E. Fr.	Kale
Descurainia sophia (L.) Webb.	Tansy mustard
Draba sp.	Dill
Eruca vesicaria (L.) Cav. (as *E. sativa* Mill)	Rocket-salad
Erysimum asperum (Nutt.) D.C.	Western wallflower
Erysimum hieraciifolium (as *Cheiranthus allanii* (D.C.) Kuntze)	Wallflower
Lberis amara L.	Rocket candytuft
Lberis umbellata L.	Globe candytuft
Lepidium sativum L.	Garden cress
Lepidium virginicum L.	Poor-man's pepper grass
Lobularia maritima (L.) Deso.	Sweet alyssum
Matthiola incana (L.) Br.	Common stock
Matthiola sp.	Stock
Nasturtium officinale Br.	Watercress
Raphanus raphanistrum L.	Wild radish
Raphanus sativus L.	Garden radish
Raphanus sativus L. var. *hortensis* Makino	Undetermined
Rorippa sylvestris (L.) Besser	Creeping yellow cress
Rorippa sp.	Yellow cress
Sisymbrium officinale (L.) Scop.	Hedge mustard
Thlaspi arvense L.	Penny cress
CUCURBITACEAE (Gourd family)	
Citrullus lanatus (Thunb) Matsum & Nakai (as *C. vulgaris* Schrad.)	Watermelon
Citrullus vulgaris var. *citroides* (*Bailey*) Mansf.	Citron
Citrullus vulgaris var. *fistulosus*	Undetermined
Cucumis anguria L.	Gherkin
Cucumis melo L.	Melon
Cucumis melo L. var. *cantaloupensis* Naud.	Undetermined
Cucumis melo L. var. *makuwa* Makino	Undetermined
Cucumis melo L. var. *reticulatus* Naud.	Muskmelon
Cucuinis melo L. var. *utilissimus*	Undetermined
Cucumis sativus L.	Cucumber

(continued)

Table 3.2.1.1 (continued)

Latin binomial	Common name
Cucurbita maxima Duchesne	Winter squash
Cucurbita moschata Duchesne	Undetermined
Cucurbita pepo L.	Pumpkin
Cucurbita pepo L. var. *melopepo* (L.) Alef.	Bush pumpkin
Cucurbita pepo L. var. *ovifera* (L.) Alef.	Yellow-flowered gourd
Lagenaria siceraria (Mol.) Standl.	Bottle gourd
Luffa cylindrica Mill. (as *L. leucantha* (Dutch.) Rasby)	Loofah
SOLANACEAE (Nightshade family)	
Capsicum annuum L.	Pepper
Capsicum frutescens L.	Tabasco
Capsicum frutescens L. cv. 'grossum'	Sweet pepper
Cyphomandra betaceae Sendt	Tree tomato
Datura stramonium L.	Jimsonweed
Datura innoxa Mill.	Angel's trumpet
Hyoscyamus niger L.	Henbane
Lycopersicon esculentum Mill.	Tomato
Nicandra physalodes (L.) Gaertn.	Apple of Peru
Nicotiana rustica L.	Wild tobacco
Nicotiana tabacum L.	Tobacco
Petunia axillaris (Lam.) BSP	Whitemoon petunia
Petunia hybrida Hort.	Garden petunia
Petunia spp.	Petunia
Physalis angulata L.	Undetermined
Physalis peruviana L.	Cape gooseberry
Schizanthus pinnatus	Butterfly flower
Schizanthus retusus Hook.	Butterfly flower
Schizanthus sp.	Butterfly flower
Schizanthus wisetonensis Hort.	Undetermined
Solanum chacoense Bitter	Undetermined
Solanum citrullifolium Br.	Melon leaf nightshade
Solanum elaeagnifolium Cav.	Silver leaf nightshade
Solanum guitoense Lam.	Lulu
Solanum melongena L.	Eggplant
Solanum melongena var. *esculentum* Nees.	Eggplant
Solanum nigrum L.	Black nightshade
Solanum torvum Swartz	Devil's fig
Solanum tuberosum L.	Potato
CHENOPODIACEAE (Goosefoot family)	
Beta vulgaris L.	Beet
Beta vulgaris L.	Sugar beet
Chenopodium album L.	Lamb's-quarters
Chenopodium album L. var. *centrorubrum* Makino	Undetermined
Spinacia oleracea L.	Spinach
CONVOLVULACEAE (Convolvulus family)	
Convolvulus arvensis L.	Field bindweed
Convolvulus sp.	Bindweed
Ipomoea batatus (L.) Lam.	Sweet potato
LILIACEAE (Lily family)	
Allium cepa L.	Onion

(continued)

Table 3.2.1.1 (continued)

Latin binomial	Common name
Allium sativum L.	Garlic
Asparagus officinalis L. var. *altilis* L.	Asparagus
Asphodelus tenuifolius Cav.	Asphodel
Lilium candidum L.	Madonna lily
Lilium longiflorum Thurb.	Trumpet lily
Lilium sp.	Lily
Trillium foetidissimum Freeman	Red lily
Tulipa gesneriana L.	Tulip
Tulipa sp.	Tulip
APIACEAE (Parsley family)	
Aegopodium spp.	Goutweed
Anethum graveolens L.	Dill
Angelica archangelica L.	Angelica
Apium graveolens L. var. *dulce* (Mill.) Pers.	Celery
Apium graveolens L. var. *rupaceum* (Mill.) Gaud. Beaup.	Celeriac
Carum carvi L.	Caraway
Conium maculatum L.	Poison hemlock
Coriandrum sativum L.	Coriander
Daucus carota L.	Carrot
Foeniculum vulgare Mill.	Fennel
Foeniculum vulgare Mill. var. *dulce* Batt.& Trab.	Fennel
Pastinaca sativa L.	Parsnip
Petroselinum crispum (Mill.) Nym. ex Hill	Parsley
Pimpinella anisum L.	Anise
Pimpinella sp.	Undetermined
LINACEAE (Flax family)	
Linum flavum L.	Golden flax
Linum usitatissimum L.	Common flax
MALVACEAE (Mallow family)	
Abelmoschus esculentus (L.) Moench.	Okra
Abutilon theophrasti Medick	Velvetleaf
Alcea ficifolia (L.) Cav.	Antwerp hollyhock
Alcea rosea L.	Hollyhock
Gossypium hirsutum L.	Upland cotton
Gossypium sp.	Cotton
Hibiscus cannabinus L.	Indian hemp
Hibiscus rosa-sinensis L.	Chinese hibiscus
Hibiscus sabdariffa L.	Jamaica sorrel
Lliamna rivularis (Dougi.) Greene	Undetermined
Lavatera arborea L.	Tree mallow
Malvaviscus arboreus Cay.	Wax mallow
Malvaviscus sp.	Sleepy mallow
PEDALIACEAE (Pedalium family)	
Sesamum indicum L.	Sesame
ROSACEAE (Rose family)	
Fragaria ananassa Duchesne (as *F. chiloensis* (L.) Duchesne var. *ananassa* Bailey	Strawberry

(continued)

Table 3.2.1.1 (continued)

Latin binomial	Common name
Fragaria sp.	Strawberry
Malus sylvestris Mill. (as *M. domestica* Borkh.) (or *M. pumila* Mill.)	Apple
Malus sp.	Apple
Prunus americana Marsh.	American plum
Prunus amygdalus Batsch	Almond
Prunus armeniaca L.	Apricot
Prunus domestica L.	Garden plum
Prunus persica (L.) Batsch	Peach
Prunus sp.	Undetermine
Pyrus communis L.	Pear
Rosa sp.	Rose
Rubus sp.	Raspberry
RUTACEAE (Rue family)	
Citrus aurantifolia Christm.	Lime
Citrus aurantifolia Christm. var. *dulcis*	Lime
Citrus aurantium L.	Seville orange
Citrus latifolia Tanaka	Persian lime
Citrus limon Burm. (as *C. medica* L. var. *limonum)*	Citron
Citrus maxima (Burm.) Merril (as *C. grandis* (L.) Osbeck)	Pummelo
Citrus medica L. (as *C. medica* L. var. *ethrog* Engl)	Citron
Citrus paradisi Macfady	Grapefruit
Citrus reticulata Blanco orange	Mandarin orange tree/Mandarin
Citrus sinensis Osbeck.	Sweet orange
Citrus spp.	Undetermined
VITACEAE (Vine family)	
Vitis vinifera L.	European wine grape
MORACEAE (Mulberry family)	
Ficus carica L.	Fig
Ficus magnifolia Muell	Magnolia-leaf fig
Morus alba L.	White mulberry
Morus spp.	Mulberry
EUPHORBIACEAE (Spurge family)	
Euphorbia dentata Michx.	Toothed spurge
Euphorbia pulcherrima Willd. ex. Klotzsch	Pointsettia
Euphorbia serphyllifolia Pers.	Thyme-leaved spurge
Euphorbia spp.	Undetermined
Ricinus communis L.	Castor bean
Scabiosa sp.	Sweet scabious
MUSACEAE (Banana family)	
Musa paradisiaca L.	Edible plantain
Musa spp.	Banana
ANNONACEAE (Custard-Apple family)	
Annona squamosa L.	Sugar apple

(continued)

Table 3.2.1.1 (continued)

Latin binomial	Common name
Asimina sp. Adans	Pawpaw
PINACEAE (Pine family)	
Chamaecyparis lawsoniana (Murr.) Parl.	Lawson white cedar
Cryptomeria japonica (L f.) Don.	Japanese cedar
Larix kaempferi (Lamb.) Carriere	Japanese larch
Pinus densiflora Slebd.& Zucc.	Japanese red pine
IRIDACEAE (Iris family)	
Freesia spp. Eckl. Ex *Klatt*.	Undetermined
Gladiolus spp.	Gladiolus
Iris spp.	Iris
Iris xiphioides Ehrh.	English iris
CAMPANULACEAE (Bluebell family)	
Campanula medium L.	Canterbury bells
Campanula persicifolia L.	Willow bellflower
Campanula pyrimidalis L.	Undetermined
Campanula rapunculoides L.	Creeping bellflower
Campanula sp.	Undetermined
Lobelia erinus L.	Edging lobelia
CANNABACEAE (Hemp family)	
Cannabis sativa L.	Marijuana, hemp
Humulus lupulus L.	Common hop
Humulus sp.	Hop
LAMIACEAE (Mint family)	
Lamium amplexicaule L.	Henbit
Lamium spp.	Deadnettle
Mentha piperita L.	Peppermint
Mollucella laevis L.	Bells of Ireland
Ocimum basilicum L.	Basil
Perilla frutescens (L.) Britt. var. *japonica* (Hassk.) Hara	Undetermined
Physostegia virginiana (L.) Benth.	Obedience
Salvia sp.	Sage
Solenostemon scutellarioides (L.) Codd.	Coleus
Stachys floridana Shuttleworth ex. Benth.	Undetermined
PAPAVERACEAE (Poppy family)	
Argemone sp.	Argemony
Eschscholzia californica Cham.	California poppy
Eschscholzia spp.	California poppy
Glaucium flavum Crantz.	Undetermined
Papaver somniferum Crantz.	Opium poppy
Romneya sp.	Matilija poppy
PASSIFLORACEAE (Passionflower family)	
Passiflora edulis Sims	Purple granadilla
Passiflora sp.	Passion fruit
PLANTAGINACEAE (Plantain family)	
Plantago lanceolata L.	Buckhorn
POLEMONIACEAE (Polemonium family)	
Phlox drummondii Hook	Annual phlox

(continued)

Table 3.2.1.1 (continued)

Latin binomial	Common name
Phlox sp.	Phlox
POLYGONACEAE (Buckwheat family)	
Fagopyrum esculentum Moench.	Buckwheat
Fagopyrum tataricum Gaertn.	Buckwheat
Rheum rhaponticum L. (as *R. officinale* Baill.)	Rhubarb
Rumex crispus L.	Yellow dock
PORTULACACEAE (Purslane family)	
Portulaca oleraceae L.	Common purslane
PRIMULACEAE (Primrose family)	
Anagallis arvensis L.	Scarlet pimpernel
MARTYNIACEAE (Martynea family)	
Proboscidea louisianica (Mill.) Thell.	Probiscus flower
ASTERACEAE (Aster family)	
Acroptilon repens (as *Centaurea repens* L.)	Russian knapweed
Ageratum conyzoides L.	Undetermined
Ambrosia artemisiifolia L.	Ragweed
Ambrosia hispida Pursh	Undetermined
Arctium minus (Hill) Bernh.	Common burdock
Arctium lappa L.	Great burdock
Arctotis stoechadifolia Bergius	African daisy
Aster sp.	Aster
Bellis perennis L.	English daisy
Bidens biternata (Lour.) Merr. & Scherff.	Undetermined
Brachycombe iberidifolia Benth.	Swan River daisy
Calendula officinalis L.	Pot marigold
Callistephus chinensis (L.) Hees	China-aster
Carthamus tinctorius L.	Safflower
Centaurea cyanus L.	Bachelor's button
Centaurea dealbata Willd.	Undetermined
Centaurea diffusa Lam.	Diffuse knapweed
Centaurea montana L.	Mountain bluet
Centaurea moschata L. (as *C. oderata* Hort.)	Sweet sultan
Centaurea solstitialis L.	Yellow star-thistle
Centaurea sp.	Knapweed
Chrysanthemum cinerariifolium (Trever) viz.	Dalmatian pyrethrum
Chrysanthemum coccineum Willd.	Common pyrethrum
Chrysanthemum coronarium L. var. *spatiosum*	Undetermined
Chrysanthemum leucanthemum L. var. *pinnatifidum* Lecoq. & Lamotte	Ox-eye daisy
Chrysanthemum maximum Ramond	Daisy
Chrysanthemum morifolium Ramat.	Florists' chrysanthemum
Chrysanthemum spp.	Chrysanthemum
Cichorium endivia L.	Endive
Cichorium intybus L.	Chicory
Cirsium arvense (L.) Scop.	Canada Thistle
Cirsium vulgare (Savi) Ten. (as *C. lanceolatum* (L.) Juk.	Bull thistle

(continued)

Table 3.2.1.1 (continued)

Latin binomial	Common name
Cnicus arvensis Hoffm. (as *Carduus arvensis* (L.) Robs.)	Thistle
Cnicus benedictus L.	Blessed thistle
Cnicus spp.	Blessed thistle
Coreopsis grandiflora Hagg. ex Sweet1969	Tickseed
Coreopsis spp.	Tickseed
Coreopsis stillmani (Gray) Blake	Tickseed
Coreopsis tinctoria Nutt.	Calliopsis
Cosmos bipinnatus Cay.	Cosmos
Crepis japonica (L.) Benth. (as *Youngia japonica* (L.) D.C.)	Hawk's beard
Cynara scolymus L.	Artichoke
Dahlia pinnata Cav. (as *D. variabilis* (Willd.) Desf.)	Garden dahlia
Dahlia pluvialis (L.) Moench.	Undetermined
Dahlia spp.	Dahlia
Dimorphotheca aurantiaca D.C.	Cape marigold
Dimorphotheca sp. Moench.	Cape marigold
Erechtites hieracifolia (L.) Raf.	Pilewort
Erigeron annuus (L.) Pers.	Sweet scabious
Erigeron canadensis L.	Hog-weed
Gaillardia pulchella Foug.	Firewheel
Gaillardia sp.	Gaillardia
Galinsoga parviflora Cav.	Small-flowered galinsoga
Gazania rigens (L.) Gaertn.	Treasure flower
Gerbera jamesonii Bolus	Transvaal daisy
Gerbera spp.	Gerbera
Gnaphalium purpureum L.	Purple cudweed
Gynura sp.	Undetermined
Helianthus annuus L.	Sunflower
Helianthus tuberosus L.	Jerusalem artichoke
Helichrysum bracteatum (Venten.) Andr.	Undetermined
Helichrysum sp.	Strawflower
Helipterum almcans (Cunn.) D.C. (as *Acroclinum album* Gray)	Everlasting
Helipterum roseum (Hook.) Benth. (as *Acroclinum roseum* Hook.)	Paper flower
Ixeris dentata (Thunb) Nakai	Undetermined
Iva xanthifolia Nutt.	False ragweed
Iva sp.	Marsh-elder
Lactuca sativa L.	Garden lettuce
Lactuca sativa var. *capitata* L.	Head lettuce
Lactuca sativa var. *crispa* L.	Leaf lettuce
Lactuca sativa var. *longifolia* Lam.	Romaine lettuce
Lactuca serriola L.	Prickly lettuce
Lactuca spp.	Lettuce
Liatris sp.	Gay-feather
Onopordum acanthium L. (as *Cnicus lanceolatus* L.)	Scotch thistle
Osteospermum ecklonis (D.C.) Norl.	African daisy
Osteospermum fruiticosum (L.) Norl.	African daisy

(continued)

Table 3.2.1.1 (continued)

Latin binomial	Common name
Parthenium argentatum Gray	Guayule
Rudbeckia laciniata (L.) Per.	Coneflower
Rudbeckia laciniata (L.) var. *hortensis* Bailey	Golden-glow
Scorzonera hispanica L.	Black salsify
Scorzonera sp.	Salsify
Senecio cruentus (Masson) D.C.	Florists' cineraria
Senecio spp.	Groundsel
Senecio vulgaris L.	Groundsel
Silybum marianum (L.) Gaertn.	Milk thistle
Solidago canadensis L. var. *salebrosa* (Piper) Jones	Goldenrod
Sonchus arvensis L.	Field sow-thistle
Sonchus asper (L.) Hill.	Spiny sow-thistle
Sonchus oleraceus L.	Common sow-thistle
Sonchus spp.	Sow-thistle
Stokesia laevis (Hill.) Greene	Stokes' aster
Tagetes erecta L.	African marigold
Tagetes patula L. (as *Tanmpteises patula* L.)	African marigold
Tagetes sp.	Marigold
Tagetes tenuifolia Cav.	Signet marigold
Taraxacum kok-saghyz Rodin.	Russian dandelion
Taraxacum officinale Wiggers	Common dandelion
Tragopogon porrifolius L.	Salsify
Venidium decurrens Less.	Undetermined
Venidium fastiuosum (Jacq.) Stapf.	Cape daisy
Verbesina spp.	Crown-beard
Xanthium pensylvanicum Wallr.	Cocklebur
Zinnia elegans Jacq. (*Z. elegans* L.)	Zinnia
Zinnia spp.	Zinnia
BEGONIACEAE (Begonia family)	
Begonia tuberhybrida Voss	Begonia
BERBERIDACEAE (Barberry family)	
Berberis sp.	Barberry
BORAGINACEAE (Borage family)	
Anchusa azurea Mill.	Alkanet (Buglossum)
Anchusa capensis Thunb.	Bugloss
Cynoglossum amabile Stapf. & Drumm.	Chinese forget-me-not
Mertensia lanceolata (Pursh.) D.C.	Bluebell
Myosotis arvensis (L.) Hill	Forget-me-not
Myosotis scorpiodes L.	Forget-me-not
Myosotis sp.	Forget-me-not
Myosotis sylvatica Hoffm. (as *M. oblongata* Link.)	Garden forget-me-not
RANUNCULACEAE (Crowfoot family)	
Aconitum carmichaelii Debeaux	Azure monkshood
Anemone coronaria L.	Poppy anemone
Aquilegia vulgaris L.	European crowfoot
Aquilegia spp.	Columbine

(continued)

Table 3.2.1.1 (continued)

Latin binomial	Common name
Consolida orientalis (Gray) Schrod. (as *Delphinium ajacis* L.)	Rocket larkspur
Delphinium cheilanthum Fisch.	Garland larkspur
Delphinium cultorum Voss	Larkspur
Delphinium elatum L.	Candle larkspur
Delphinium grandiflorum L.	Bouquet larkspur
Delphinium sp.	Larkspur
Ranunculus asiaticus L.	Persian buttercup
Ranunculus sp.	Buttercup
Trollius sp.	Globeflower
SCROPHULARIACEAE (Figwort family)	
Antirrhinum majus L.	Common snapdragon
Calceolaria crenatiflora Cav.	Slipperwort
Calceolaria sp.	Slipperwort
Digitalis purpurea L.	Common foxglove
Linaria canadensis (L.) Dum	Blue toadflax
Linaria spp.	Toadflax
Linaria vulgaris Mill.	Butter-and-eggs
Nemesia sp.	Undetermined
Paulownia sp.	Undetermined
Verbascum blattaria L.	Moth mullein
THEACEAE (Camellia family)	
Camellia japonica L.	Camellia
Camellia sp.	Camellia
TILIACEAE (Basswood family)	
Corchoris capsularis L.	Jute
TROPAEOLACEAE (Tropaeolum family)	
Tropaeolum majus L.	Garden nasturtium
Tropaeolum sp.	Nasturtium
URTICACEAE (Nettle family)	
Urtica dioica L. ssp. *gracilis* (Ait.) Selander var. *gracilis*	Stinging nettle
Urtica gracilis Ait.	Nettle
Urtica sp.	Nettle
VALERIANACEAE (Valerian family)	
Valeriana officinalis L.	Common valerian
VIOLACEAE (Violet family)	
Viola odorata L.	Sweet violet
Viola sp.	Pansy
ARACEAE (Arum family)	
Epipremnum aureum (Linden & Andre) Bunt.	Pothos
Philodendron scandens Koch & Sello	Heart-leaf philodendron
Philodendron selloum C. Koch	Philodendron
ACANTHACEAE (Acanthus family)	
Hemigraphis alternata (Bum.) Anderson	Red ivy
ACTINIDIACEAE (Actinidia family	
Actinidia chinensis Planch.	Chinese gooseberry

(continued)

Table 3.2.1.1 (continued)

Latin binomial	Common name
AIZOACEAE (Carpet-weed family)	
Dorotheanthus belliformis (Burm.)	Livingstone daisy
Tetragonia tetragonioides (Pall.) Kuntze (as *T. expansa* Murr.)	New Zealand spinach
AMARANTHACEAE (Amaranth family)	
Amaranthus retroflexus L.	Redroot pigweed
Amaranthus sp.	Undetermined
Celosia sp.	Cock's-comb
APOCYNACEAE (Dogbane family)	
Latin binomial	Common name
Catharanthus roseus (L.) Don (as *Vinca rosea* L.)	Madagascar periwinkle
Vinca minor L.	Common periwinkle
ARALIACEAE (Ginseng family)	
Aralia cordata Thurb.	Spikenard
Hedera helix L.	English ivy
Panax pseudoginseng Wallich	Ginseng
Panax quinquefolius L.	American ginseng
Schefflera arboricola Hayata	Undetermined
ARISTOLOCHIACEAE (Birthwort family)	
Aristolochia durior Hill	Dutchman's pipe
Asarum canadense L.	Wild ginger
ASCLEPIADACEAE (Milkweed family)	
Asclepias sp.	Milkweed
Stephanotis floribunda Brongn.	Undetermined
CAPPARIDACEAE (Caper family)	
Cleome viscosa L.	Tickweed
CARYOPHYLLACEAE (Pink family)	
Dianthus caryophyllus L.	Carnation
Gypsophila paniculata L.	Baby's-breath
Stellaria media (L.) Cyr.	Chickweed
CELASTRACEAE (Staff-tree family)	
Euonymus alata (Thunb.) Siebold	Winged spindle-tree
Euonymus alata (Thunb.) Siebold var. *compacta*	Winged spindle-tree
CYPERACEAE (Sedge family)	
Cyperus rotundus L.	Nut grass
DIPSACACEAE (Teasel family)	
Dipsacus fullonum L. (as *D. sylvestris* Huds.)	Common teasel
FAGACEAE (Beech family)	
Quercus sp.	Oak
FUMARIACEAE (Fumitory family)	
Dicentra spectabilis (L.) Lem.	Bleeding heart
GENTIANACEAE (Gentian family)	
Gentiana lutea L.	Yellow gentian

(continued)

Table 3.2.1.1 (continued)

Latin binomial	Common name
GERANIACEAE (Geranium family)	
Pelargonium hortorum Bailey	Zonal geranium
Pelargonium spp.	Pelargonium
GESNERIACEAE (Gesneria family)	
Sinningia speciosa (Lodd.) Hiern.	Gloxinia
HIPPOCASTANACEAE (Horse Chestnut family)	
Sculus hippocastanum L.	Horse chestnut
HYDRANGEACEAE (Hydrangea family)	
Hydrangea sp.	Hydrangea
JUGLANDACEAE (Walnut family)	
Engelhardtia spicata Blume	Undetermined
LAURACEAE (Laural family)	
Persea americana Mill.	Avacado
Persea borbonia (L.) Spreng	Laureltree
MYRSINACEAE (Myrsine family)	
Ardisia crenta Sims	Coralberry
Ardisia crispa (Thunb.) A.DC	Ardisia
MYRTACEAE (Myrtle family)	
Eucalyptus sp.	Undetermined
OLEACEAE (Olive family)	
Forsythia sp.	Golden-bells
Forsythia suspensa (Thunb.) Vahl.	Golden-bells
Forsythia viridissima Lindl.	Golden-bells
Syringa vulgaris L.	Common lilac
ONAGRACEAE (Primrose family)	
Fuchsia sp.	Undetermined
Oenothera sp.	Evening primrose
OROBANCHACEAE (Broom-rape family)	
Orobanche cernua Loefl.	Broomrape
Orobanche spp.	Broomrape
PAEONIACEAE (Paeony family)	
Paeonia lactiflora Pall.	Garden peony
Paeonia officinalis L.	Peony
POLYPODIACEAE (Fern family)	
Rumohra adaintiformis (G. Forst.) Ching	Leather leaf fern
SALVINIACEAE (Salvinia family)	
Azolla pinnata R. Br.	Mosquito- fern

Table 3.2.1.2 Additions in host range of *Sclerotinia sclerotiorum* since 1990

Host scientific name	English name	Reference
Abelmoschus moschatus Medic	Muskdana	Singh et al., 2000
Abutilon theophrasti	Velvet leaf	Dillard et al., 1991; Jurkovic and Culek, 1997
Acca sellowiana		Aloj et al., 1994
Amaranthus deflexus		Phillips, 1992
Amaranthus viridis		Ghasolia et al., 2004a
Ambrosia artemisiifolia	Ragweed	Bohar and Kiss, 1999
Ammi maju	Bishop's weed	Upadhyaya, 1994; Kanno and Ohkubo, 1999
Anethrum sowa		Sinha and Singh, 1991
Angelica keiskei	Beard tongue	Takeuchi and Horie, 1996
Anoectochilus lanceolatus	Jewel Orchid	Bag, 2006
Anthriscus cerefolium		Koike, 1999
Apios americana		Holcomb, 1990
Artemisia scupoonia		Ghasolia et al., 2004a
Aster pilosus		Takeuchi and Horie, 1999
Azadirachta indica		Ghasolia and Shivpuri, 2004
Basella rubra (*B. alba*).		Kanno and Ohkubo, 1999
Bidens formosa		Phillips, 1992
Bidens pilosa		Phillips, 1992
Borago officinalis	Borage	Bradley et al., 2005
Bouvrdia sp.		Horie and Hoshi, 2002
Brassica arvensis		Ghasolia et al., 2004a
Brassica carinata		Corato and Baviello, 2000
Bupleurum rotundifolium	Bupleurum	Togawa, 2000
Calendula officinalis		Minuto et al., 2001
Campanula carpatica		Garibaldi et al., 2002b
Centaurea cyanus		Sinha and Singh, 1991
Chrysanthemoides monilifera sp. ssp. *Rotundata*	Batou bush	Cother, 2000
Chrysanthemum morifolium		Wright and Palmucci, 2003
Cicer arietinum	Chickpea	Chen et al., 2006
Citrus unshiu		Song and Koh, 1999
Cleome spinosa		Liu-Xue Min et al., 2002
Coriandrum sativum	Coriander	Gaetan et al., 1997
Craspedia globosa		Wolcan and Grego, 2005
Cynara scolymus	Globe artichoke	Granata and Tirro, 1989
Cynoglossum officinale	Houndstongue	Huang et al., 2005b
Datura stramonium		Ghasolia et al., 2004a

(continued)

Table 3.2.1.2 (continued)

Host scientific name	English name	Reference
Diplotaxis tenuifolia	Wild rocket	Minuto et al., 2005a; Garibaldi et al., 2005
Didiscus caeruleus		Kanno and Ohkubo, 1999
Diplotaxis tenuifolia		Garibaldi et al., 2005
Echinacea purpurea	Cone flower	Chang et al., 1997b
Echium vulgare	Echium	Rio et al., 2005
Eclipta prostrata		Melouk et al., 1992
Eureka lemin	Lemon	Fogliata et al., 1999
Eustoma grandiflora	Lisianthus	Wolcan et al., 1996
Fagopyrum esculentum	Buckwheat	Mondal et al., 2003
Fagopyrum tatarum	Buckwheat	Mondal, 2004
Felicia amelloides Gaertn	Blue Marguerite	Minuto et al., 2004a; Garibaldi et al., 2004; Wright et al., 2005
Gaillardia grandiflora		Koike, 1997
Gazania spp.	Gazania	Garibaldi et al., 2001; Wolcan, 2004
Gazania hybrid		Garibaldi et al., 2002a
Gerbera jamesonii	African daisy	Matheron and Matejka, 1994; Wolcan, 2004
Goodyera schlechtendaliana	Jewel orchid	Bag, 2006
Gossypium sp.		Charchar et al., 1999
Guizotia abyssinica	Niger	Bradley et al., 2003
Heliotropium ellipticum		Ghasolia et al., 2004a
Helichrysum bracteatum		Takeuchi and Horie, 1999
Heracleum mantegazzianum	Giant hogweed	Erneberg et al., 2003
Hibiscus cannabinus		Corato, 1996
Hibiscus sp.	Hemp.	Bains et al., 2000
Holoptelea integrifolia		Ghasolia et al., 2004a
Hutchinsia alpina		Minuto et al., 2004a
Lagenaria siceraria		Pandey and Pandey, 2002
Lapsana apogonoides		Kanno and Ohkubo, 1999
Lathyrus sativus		Zimmer and Campbell, 1990
Leonurus sibiricus		Kwon and Park 2002
Linum usitatissimum	Flax	Rashid, 2001
Lupinus texensis		Woodard and Newman, 1993
Ocimum basilicum	Basil	Paulitz, 1997
Osteospermum sp.	African daisy	Wright et al., 2005; Holcomb, 2005
Papaver somniferum	Opium poppy	Singh et al., 2003
Penstemon sp.		Takeuchi and Horie et al., 1996
Perilla frutescens	Perilla	Togawa, 2000
Petunia hybrida		Holcomb, 2001

(continued)

Table 3.2.1.2 (continued)

Host scientific name	English name	Reference
Phacelia tanacetifolia		Ivancia, 1993
Phaseolus vulgaris		Gupta et al., 1997
Phyllanthus fraternus		Ghasolia et al., 2004a
Pisum sativum		Iqbal et al., 1998
Portulaca oleracea		Kanno and Ohkubo, 1999
Ranunculus asiaticus	Persian buttercup	Wright et al., 2005; Garibaldi et al., 2003
Ranunculus glacialus		Graf and Schumachar, 1995
Rosmarinus officinalis	Rosemary	Mohan, 1994; Minuto et al., 2005b
Salvia coccinea		Rajappan et al., 1999
Salvia officinalis	Common saga	Minuto et al., 2004b
Salvia reflexa	Salvia	Jons et al., 1996
Schizanthus wisetonensis	Butterfly flower	Bag, 2003a; Garibaldi et al., 2002b
Silene vulgaris	Bladder campion	Takeuchi and Horie, 1996
Stevia rebaudiana	Stevia	Chang et al., 1997a
Tagetes minuta		Phillips, 1992
Trachellium caeruleum		Wolcan and Grego, 2005
Trachyspermum ammi	Bishop weed	Singh and Singh, 2001
Trianthema portulacastrum		Ghasolia et al., 2004a
Thymus citriodorus	Thyme	Minuto et al., 2004b
Tridax procumbens		Ghasolia et al., 2004a
Trillium foetidissimum		Holcomb, 1990
Verbena officinalis		Takeuchi and Horie, 1996
Vernonia cinerea		Ghasolia et al., 2004a
Vitis vinifera	Grape	Hall et al., 2002
Vitis sp.		Latorre and Guerrero, 2001
Vitis vinifera ssp. Roptundata		Hall et al., 2002
Withania somnifera		Ghasolia et al., 2004a

Table 3.2.2.1 Additions in host range of *Sclerotinia minor* since 1990

Host scientific name	English name	Reference
Allium vineale	Wild garlic	Hollowell and Shew, 2005a
Arabidopsis thaliana	Mouse-ear cress	Hollowell et al., 2003
Artemisia dracunculus		Gaetan and Madia, 1995
Artemisia dracunculoides		Gaetan and Madia, 1995
Austrian winter		Koike et al., 1996
Brassica kaber	Wild mustard	Hollowell et al., 2003
Brassica oleracea var *botrytis*	Cauliflower	Koike et al., 1994
Capsimum annuum	Bell pepper	Gonzalez et al., 1998
Cardamine parviflora	Bittercress	Hollowell et al., 2003
Cerastium vulgatum	Mouse-ear	Hollowell et al., 2003

(continued)

Table 3.2.2.1 (continued)

Host scientific name	English name	Reference
Cerastium fontanum	Chickweed	Hollowell et al., 2003
Cicer arietinum	Chickpea	Gonzalez et al., 2003; Fuhlbohm et al., 2003; Matheron and Porchas, 2000
Cichorium intybus	Radicchio	Koike and Subbarao, 1995
Conyza canadensis	Horseweed	Hollowell et al., 2003
Coronopus didymus	Swinecress	Hollowell et al., 2003
Cyperus esculentus	Yellow nutsedge	Hollowell and Shew, 2001
Diocus carrota	Carrot	Kora et al., 2002
Geranium carolinianum		Hollowell and Shew, 2004
Helianthus annuus	Sunflower	Molinero Ruiz and Malero Vara, 2002
Lamium amplexicaule	Henbet	Hollowell et al., 2003
Lens culinaris	Lentil	Gonzalez et al., 2003; Fuhlbohm et al., 2003
Ocimum basillium	Basil	Koike and Brien, 1995
Orchis laxiflora	Orchid	Eken et al., 2003
Orchis palustris	Orchid	Eken et al., 2005
Phacelia tanacetifolia		Koike and Brien, 1995
Sida spinosa	Priekly sida	Hollowell and Shew, 2005b
Stellaria media	Common chickweed	Hollowell et al., 2003
Valerianella locusta	Corn salad	Koike, 2003
Vicia dosyearpa(*V. villasa*)		Koike and Brien, 1995

it is known only on forage legumes hence appears to be very little host specificity. There are about 250 described species of the genus (*Trifoliorum*) which are attacked by this fungus. The fungus can attack various species of Alfalfa (*Madicago sativa* L.), *Madicago falcata* L., *Medicago hispide* Gaertn., several species of white sweet clover *Melilotus alba* Desv., yellow sweet clover, *M. officinalis* (L.) Lam. and *M. indica* All., four species of clover (*Trifoliorum* spp) i.e., red clover (*T. pratense* L.), white clover including Ladino (*T. repens* L.), alsike (*T. hybridum* L.), crimson clover (*T. incarnatum* L.), berseem clover or Egyptian clover (*T. alexandrinum* L.), crownvetch and arrowleaf clover. Some of the authors have included *Sclerotinia minor* Jagger and *S. trifoliorum* Erikss. in *S. sclerotiorum*. So reports on the host range of *S. trifoliorum* are similar to those caused by *S. trifoliorum*, however *S. trifoliorum* is restricted to the forage legumes as mentioned above. Approximately 100 hosts have been recorded for this species but there is lack of conclusive evidence that *S. trifoliorum* and not another *Sclerotinia* species. The other important hosts are faba beans (*Vicia faba* L.), *Vicia sativa*, *Trifoliorum diffusum* Ehrh., *Anthyllis vulneraria*, and *Lathyrus* spp. (Dickson, 1956; Mc Gimpsey and Merser, 1984; Rowe, 1990; Singh and Singh, 1993; Dabkeviciene and Dabkevicus, 2005; Lithourgidis et al., 2007).

Chapter 4
Economic Importance

4.1 General

Yield losses due to *Sclerotinia* diseases in susceptible crops vary and may be as high as 100 per cent (Purdy, 1979). In vegetable and fruit crops, losses are due to the infected produce rotting in the field before harvest and also due to rotting during storage (Walker, 1969; Willetts and Wong, 1980). In seed crops, yields are reduced by both as reduction in seed size from the premature ripening of infected plants (Kruger, 1973, 1975b; Morrall et al., 1976) and by the loss of seed during harvesting. Seed loss is attributable to the replacement of seed by sclerotia in crops like sunflower and beans. The shattering of prematurely ripened seed pods before harvest and loss of quality in the form of smaller, shrunken and chaffy seed in crops like rapeseed and sunflower has been observed (Kruger, 1973, 1980; Morrall et al., 1976). The fungal sclerotia may be mixed with seeds of sunflower, rapeseed and beans (Hoes and Huang, 1976; Kruger et. al., 1981).

Losses of some crops from diseases caused by *Sclerotinia sclerotiorum* and other species of *Sclerotinia* are millions of dollars annually. The losses are directly from loss of yield and indirectly from lessened quality (loss in grade). There are no data to illustrate the loss of expenditures for attempts to control diseases caused by *S. sclerotiorum*, when attempts are either effective or non effective. There also is another form of loss caused by this pathogen, the production lost due to abandonment of fields for growing preferred crops for less lucrative ones, or to non crop plants, weeds or fallow (Purdy, 1979).

4.2 Peanut

In peanut under low temperature conditions, the disease can be quite destructive. More than 50 per cent loss in yield due to the disease has been reported under U.S. conditions (Beute et al., 1975; Porter et al., 1977). The severity of disease as detected in infrared imagery can be correlated with actual pod losses in the field (Porter et al., 1975; Powell et al., 1976; Cobb et al., 1977). Pod yield losses have

G.S. Saharan, Naresh Mehta, *Sclerotinia Diseases of Crop Plants: Biology, Ecology and Disease Management*,

been correlated with aggravated disease incidence resulting from damage done to the plant. The yield of the peanut crop has been recorded as 1,736 kg/ha in damaged and affected rows as against 2,658 kg/ha in healthy crop (Porter, 1980b). In Argentina, in the fields where sequencing of crops has been the same during the last 15 years, blight varied from 5 to 45 per cent and yield losses between 100 and 870 kg/ha (Marinelli et al., 1998).

4.3 Beans

One per cent of a green bean crop infection by *S. sclerotiorum*, reduces yield by 0.2–0.8 per cent depending on the severity of infection. A heavy infection of white mould is of serious concern to both growers and processors because besides reducing yield, it seriously disrupts the processing operation (Wong, 1978). In dry, edible bean plants irrigated fields in western Nebraska, 30 per cent white mould infection caused 13 per cent loss in yield. Plants severely infected with *S. sclerotiorum* sustained a mean decrease in yield of 44 per cent when compared to healthy plants. Total seed yield and the components of the yield including the seeds per plant, 100 seed weight, and number of pods per plant is significantly reduced by *S. sclerotiorum* (Table 4.3.1). Reduced number of seeds per plant is the major component of yield loss followed by reduced weight of 100 seeds. Reduced seed size account for nearly one third of the yield loss in diseased plants (Kerr et al., 1978).

4.4 Sunflower

In Manitoba, Canada during the year 1971 and 1975, more than 90 per cent sunflower plants were observed to be infected with wilt and head rot (Dueck, 1979). On an average about 40 per cent of the plants were reported to have been killed in commercial fields due to *Sclerotinia* wilt (Hoes and Huang, 1976). Dorrell and Huang (1978) reported that a sunflower field with 60 per cent of the plants affected by *Sclerotinia* wilt showed 37 per cent less yield. However, reduced yield of sunflower depends upon the stage of plant development when wilt occurs. It has been observed that plants infected and wilted at any stage from flowering to near maturity show significantly reduced seed yield. Seed yields are reduced more than 70 per cent when wilting occurs with in four weeks of flowering. The major reduction in yield of plants infected by the disease is due to rapid wilting and loss of leaf tissue similar to drought and defoliation. Seed quality as measured by test weight, oil and protein content is also adversely affected by the disease resulting in reduction in economic value. In Manitoba, 40 per cent of the plants were lost due to *Sclerotinia* disease (Young and Morris, 1927). In Tanzania, 30 to 40 per cent loss

Table 4.3.1 Seed yield, weight of 100 seeds and number of seeds and pods of healthy and *Sclerotinia sclerotiorum* infected dry bean plants (Adapted from publication of Kerr et al., 1978. With permission)

		Seed yield from five plants		Wt. of 100 seeds		No. of seeds per five plants		No. of pods per five plants	
Year	Sample	Mean (g)	C.V. (%)	Mean (g)	C.V. (%)	Mean (%)	C.V. (%)	Mean (%)	C.V. (%)
1970	Healthy	103	35	31	12	336	32	87	47
	Diseased	61	44	26	14	250	42	59	38
1971	Healthy	89	25	31	9	288	26	72	25
	Diseased	58	36	27	15	216	35	59	33
1972	Healthy	102	31	–	–	–	–	–	–
	Diseased	47	40	–	–	–	–	–	–
1973	Healthy	117	29	30	11	391	26	88	22
	Diseased	67	31	25	13	264	26	62	20
Mean	Healthy	103	30	31	11	338	28	82	31
Mean	Diseased	58	38	26	14	243	34	60	30
Mean reduction of diseased		44%	–	16%	–	28%	–	27%	–

in yield due to this disease has been reported (Wallace, 1944) while in Chile, losses amounting to 5 per cent of the value of the crop have been reported (Anon., 1952). In Argentina, *S. sclerotiorum* infection of sunflower caused reduction of 35 per cent in seed yield, 24 per cent fewer seeds/head, a 15 per cent decrease in 1,000 seed weight and lower oil content in plants of six sunflower hybrids (Sala et al., 1994). About 10 per cent of the plants are killed due to disease in North parts of India (Kolte and Mukhopadhyay, 1973), resulting in a direct loss of 10 per cent of the crop due to disease (Kolte and Tewari, 1977).

The effect of sunflower head rot on impurities in the harvested product (HP), oil content (OC) and oil quality (OA) at different levels of disease incidence (DI) has been analyzed by Aguero et al. (2001). With 100 per cent DI, seed represented 65 per cent and sclerotia 25 per cent of the HP. At greater DI levels, OC decreases and OA increases. Content of seed in the HP defines OC. Sclerotial content is the main reason for the increase in OA. At 100 per cent DI, seed + sclerotial samples yield 31 per cent less OC and 53 per cent more OA than the seed samples.

Sclerotinia head rot epidemic on sunflower crop has been valued at $100 million in USA during the year 1999 (Anon., 2005b). In Jelin, Heilingjiang and Inner Mongolia during 1986, *Sclerotinia* head rot of sunflower caused 45 per cent reduction in yield. The disease reduces 100 seed weight by 31 per cent, protein content by 12.1 per cent and oil content by 2.7 per cent. However, shell weight increases by 31.4 per cent. Germination of infected seeds reduces by 20–64 per cent (Hua et al.,

1987). *Sclerotinia* head rot causes decrease in seed oil, protein, free amino acid, sugar and phenol content and an increase in peroxidase and fatty acid content (Kumar et al., 1998).

The effect of *S. sclerotiorum* on some morphological traits of sunflower has been carried out. Results shows a decrease in plant height, stem diameter, leaf area, leaf area index, head diameter, number of filled seeds per head, seed weight per head, 1,000-kernel weight, core percentage and achene oil content and a significant increase in the diameter of the head empty area and shell content. Collar infection results in significant decrease in head diameter, number of filled seeds per head, core content, 1,000 kernel weight, seed weight per head, core content and increase in shell percentage. Stem infection results in a significant decrease in stem diameter, leaf area, leaf area index and achene oil content, while head infection results in a decrease in plant height, head diameter, number of filled seeds per head, seed weight per head, 1,000-kernel weight, core content and an increase in shell percentage (Eva and Andrei, 2000).

4.5 Rapeseed-Mustard

Due to *Sclerotinia* disease, loss estimates have been made as high as 28 per cent in individual rapeseed fields in Alberta, Canada (Morrall et al., 1976). From Saskatchewan, the yield losses due to the disease are reported to be 11.1 to 14.9 per cent (Morrall et al., 1976). The lowest yields in years of epidemic occurrence of the disease have been reported from Germany (Kruger, 1975b). In central and eastern parts of Finland, loss caused by the disease is so great that the cultivation of this crop is considered to be successful only in the southern and western areas of Finland (Jamalainen, 1954). The shattering of siliquae which mature prematurely due to the disease may be an additional yield loss factor (Morrall and Dueck, 1983). At the time of harvesting and threshing, sclerotia of the fungus get mixed with seed, and this represents an objectionable seed contaminant for export of the seeds from one country to another and thus affects the marketability of the crop. The quality of the seed is also adversely affected in partially infected plants (Kruger et al., 1981). In Nepal, grain yield, plant height, number of siliquae/plant and 1,000 grain weight is reduced with 75 per cent yield loss (Chaudhary, 1993). Rape (*B. napus*) yields decline in southern NSW, Australia from 0.39 t/ha to 1.54 t/ha due to *Sclerotinia* stem rot (Kirkegaard et al., 2006). Oil content and quality of the seed reduces in diseased plants (Aggarwal et al., 1997). In India, yield losses of 50–75 per cent have been recorded from mustard crop (Kang and Chahal, 2000; Sharma et al., 2001; Shivpuri et al., 2000). According to Shukla (2005a) that if plants are infected at or before flower initiation, results in 100 per cent yield loss where as infection after flowering stage cause more than 50 per cent yield loss. Yield losses due to *Sclerotinia* stem rot have been estimated at 5–13 per cent in North Dakota and 11.2–13.2 per cent in Minnesota, USA during 1991–1997 (Lamey et al., 1998).

4.6 Soybean

The estimated yield loss per 10 per cent disease incidence of *Sclerotinia* stem rot of soybean ranges from 83.2 to 229.0 kg/ha, with an average loss of 136 kg/ha (Danielson et al., 2004). In soybean, the model of relationship between the percentage of yield loss (Y) and the quantity of apothecia (X) is established: $Y= - 4.5499 + 2.313X$ ($r = 0.8442$). The control threshold is 3–4 apothecia per 9.75 m^2 (Pan-Hong Yu et al., 2001).

4.7 Tomato

In tomato severely infected plants show reduction in weight (84.3 per cent) and size of fruits (62.0 per cent). Nearly total loss is observed when plants are infected from early to mid bloom as compared to plants infected near harvest (Jnr et al., 2000).

4.8 Potato

In Germany, *S. sclerotiorum* causes yield reduction up to 30 per cent in potato crop in some areas of Niedersachsen (Quentin, 2004).

4.9 Pepper

An estimated 30–40 per cent loss in a 20 acre processing field in Ohio (USA) has been recorded due to *Sclerotinia* stem rot of *Capsicum annum* (Yanar et al., 1996).

4.10 Carrot

The disease affects both above and below ground portions of the carrot plant. Carrots are particularly susceptible to *S. sclerotiorum* late in growing season and during storage. Early in the growing season, the disease can occasionally cause damping off of young seedlings. Later infections of the foliage can reduce yield, by weakening the tops and rendering mechanical harvest inefficient (Geary, 1978). In Canada, 75–90 per cent crop damage has been attributed to *Sclerotinia* rot of carrot (Anon., 1931). The direct crop losses attributed to *Sclerotinia* rot of carrot in storage range from 30 per cent in Manitoba (Finlayson et al., 1989) to 50 per cent in Nova Scotia (Anon., 1970). In the United States serious losses have been recorded during carrot transportation (Rader, 1952) and storage (Hansen et al., 2001).

Chapter 5
The Disease and Symptoms

5.1 The Disease

The *Sclerotinia* diseases are known under a variety of names and the most common of these along with some of the host plants most seriously affected are the cottony rot, white mould, or watery soft rot of beans, cabbage, carrot, eggplant, citrus, peanut, potato, stock, tobacco etc.; stem rot and timber rot of cucumber, squash, beans, artichoke, asparagus, chrysanthemum, dahlia, delphinium, peony, potato, tomato, soybean, sweet potato etc.; lettuce drop, broad bean, beet, cabbage etc.; damping off of celery, lettuce, rapeseed-mustard etc.; crown rot or wilt of sunflower, safflower, columbine, snapdragon etc.; blossom blight of narcissus, camellia etc.; pink joint of red pepper, stem canker of hollyhock, root and crown rot of clover; white blight, white rot, stem blight, stalk break and stem canker of rapeseed-mustard, head rot of sunflower, safflower, castor and dollar spot of turf grass (causal fungus previously known as *Sclerotinia homoeocarpa* now considered as species of *Lenzia* and *Moellerodiscus*).

A new *Sclerotinia* sp. strain Let-19, isolated from lettuce in central China has been reported by Li, Guo-Qing et al. (1998). Results of pathogenicity tests showed that hyphae from myceliogenically germinated sclerotia readily causes infections on uninjured lettuce leaves but only causes infections of other plant species such as rapeseed on injured leaves. The fungus is not pathogenic to Chinese cabbage (*Brassica pekinensis*), radish (*Raphanus sativus*) and carrot. The optimum temperature for growth of *Sclerotinia* sp. strain Let-19 is 20°C and its growth rate is slower than *S. trifoliorum*. Sclerotia of Let-19 germinate readily to produce stipes, but the stipes rarely develops into apothecia under diffused light. Results of preliminary electrophoretic studies of soluble proteins and enzymes shows that strain Let-19 belongs to the genus *Sclerotinia* and it is distinctively different from *S. sclerotiorum* and *S. minor* which are known pathogens of lettuce drop.

G.S. Saharan, Naresh Mehta, *Sclerotinia Diseases of Crop Plants: Biology, Ecology and Disease Management*,

5.2 Symptoms

5.2.1 General

The symptoms caused by *Sclerotinia* vary somewhat with the host or host part affected and with the environmental conditions. The most obvious and typical early symptom of *Sclerotinia* diseases is the appearance on the infected plant of a white fluffy mycelial growth in which soon afterwards develop large, compact resting bodies known as sclerotia. The sclerotia are white at first but later become black and hard on the outside and may vary in size from 2 to 10 or more millimeters in diameter, although they are usually more flattened and elongated rather than spherical. Stem of infected succulent, herbaceous plants at first develop pale or dark brown lesions at their base. The lesions are often quickly covered by white cottony patches of fungal mycelium. In the early stages of lesion development in the stem, the foliage may show little sign of attack and infected plants are easily overlooked until the fungus grows completely through the stem and the stem rots. Then the foliage above the lesion wilts and dies more or less quickly. In some cases the infection may begin on a leaf and then move into the stem through the leaf. The sclerotia of the fungus may be formed either internally in the pith of the stem, giving no outward signs of their presence there, or they may be formed on the outside of the stem where they are quite apparent.

Leaves and petioles of plants such as lettuce, celery and beet suddenly collapse and die as the fungus infects the base of the stem and the lower leaves. Rapidly the fungus invades and spreads through the stem, and the entire plant dies and collapses. Each leaf dropping downward until it rests on the one below. Mycelium and sclerotia usually appear on the lower surface of the outer leaves, but under moist conditions the fungus invades the plant completely and causes it to rot, producing a white, fluffy, mycelial growth over the entire plant. If dry weather follows infection, the fungus forms cankers in the stem that kill the plant without a soft rot. Attack of celery produces a characteristic pink or reddish brown, water-soaked area at the base of the affected petioles that is often covered by the white mycelium and the rot may spread through the stalks causing the collapse of the whole plant.

Fleshy storage organs, such as carrots, infected by *Sclerotinia* develop a white, cottony growth on their surface whether they are still in the field or in storage. Black sclerotia are formed on externally invaded tissues which appear darker than healthy ones and become soft and watery. If the disease develops after harvest in the storage house, the rot spreads to adjacent roots or whatever the storage organs are available and produces pockets of rotted organs or all the organs in the crate may become infected and collapse producing a watery soft rot, covered by fungus growth.

Fleshy fruits such as cucumber, squash, eggplant and seed pods of beans, etc. are also attacked by *Sclerotinia* either through their closest point to the ground, or at the point of their contact with the ground or through their senescent flower parts. The fungus causes a wet rot that spreads from the tip of the fruit or pod to the rest

of the organ which eventually becomes completely rotted and disintegrated. The white fungal mycelium and the black sclerotia can usually be seen both externally and within the affected pods and fruits.

Flower infection is important primarily in camellias and narcissus. Few to many small, watery, light-brown spots appear on the petals. The spots may enlarge, coalesce and involve the entire petal and eventually the entire flower becomes dark brown and drops, but disintegration of the flowers occurs only after they have fallen and in wet weather, when the fungus produces abundant mycelium and sclerotia.

5.2.2 Cabbage

Sclerotinia diseases of cabbage are known under several names including watery soft rot, drop, cottony rot and when on cabbage seed plants as white blight. The earliest symptoms of the disease in seed cabbage are small, white spots on the stalk or on the base of the petioles. These lesions spread rapidly, usually girdling the entire stalk and extending along the main axis of the stalk for a distance of 4–6 in., becoming elliptical in outline. The surface of the lesion is grayish-white, hence comes the name “white blight”. The white colour is due to cottony mycelium produced by the fungus. When a stalk is completely girdled, food transport ceases and the plant dies. Very often the stalk breaks over at the girdled region, the pith tissue underlying a surface lesion is destroyed by the fungus and hard, black sclerotia which vary in size and shape are produced in the destroyed tissue. These hard, black resting bodies are usually oblong and vary from 1/8 to 1/2 in. in length, their diameter usually ranges from 1/12 to 1/4 in.. Although stalk infections are most common on the lower regions of the main stem, secondary branches are also infected. The loss in seed yield is much less than when the main stem is infected because lesions nearly always originate in the axils of the leaves and it is thought that water held in the leaf axils by the large, fleshy petioles assists the spores in establishing infections (Pound, 1946). A luxuriant white cottony mass of mycelium with the large black sclerotia embedded in it, develop on diseased cabbage heads in transit and storage. The fungus can spread from head to head while in a box or package. Cauliflower, cabbage, rutabaga and cauliflower crops grown for second year for seed can be affected by *Sclerotinia* infections on their stems. Stem lesions are long, gray-white and may girdle plants, the fungus can invade the pith and cause death before seeds are produced. This phase is called white blight (Sherf and Macnab, 1986).

5.2.3 Cauliflower

The earliest symptoms on cauliflower appear as loss of turgidity of leaves during the daytime but the leaves recover during night. Affected plants become dull white to pale yellow. The yellowing starts right from the tip of older leaves and moves to downward

till the whole leaf is involved. Such leaves shed pre-maturely. Midrib and petioles of lower leaves especially touching the soil, show dark brown to black soft rot of the leaves and fluffy growth of the fungus is also observed during cool humid weather. Rotting from the petioles advances to the stalk where dark brown to black spots are formed. The spots enlarge and girdle the stem at the ground level. Stem pith rots giving way to large caustic lined inside with fluffy mycelium and sclerotia. Affected curds show brown to dark brown rotting from the center. In seed crop, the fungus attacks the inflorescence on which mycelium and sclerotia can be seen (Singh, 1987).

5.2.4 Eggplant

The infection may occur at any part of the foliage mainly on the stem or branches. At the point of infection, a dry discoloured spot develops. It gradually girdles the entire stem and also progresses up and down. As a result of tissue necrosis, the portion of the plant beyond the point of infection wilts. If the infection is at the base of the main stem, the entire plant wilts, if it occurs on branches, partial wilting occurs. Fluffy white mycelial mats on infected tissues of stem, leaves and fruit with dark sclerotia of irregular shape and size can be observed. On opening the dry portion of the stem, pith can be seen full of fungal sclerotia which may be small or large, elongated or cylindrical and often attached to each other end to end. These sclerotia may also develop on the main stem along with white mycelium of the fungus sticking to host surface. The sclerotia are brown to black. When fruits are attacked, there is rotten of the flesh and in the rotting tissues large number of sclerotia of the fungus can be seen (Singh, 1987; Iqbal et al., 2003).

5.2.5 Tomato

On stems of tomato, potato, cucumber and beans, the symptoms occur either on the main stem or on secondary branches, beginning in the crotches or at points of injury, especially at ground level. Water-soaked areas develop and become light to dark brown. A white mass of mycelium with embedded black sclerotia appears on the stem surface and in the pith. Soft rot may follow, however, if conditions turn warm and dry, a dry canker may develop and kill the plant without soft rot developing (Sherf and Macnab, 1986).

5.2.6 Vegetable Crops

Growing plants are attacked at any time from the seedling stage to maturity. On some hosts, the pathogen infects the main stem somewhere near the soil line

invading the cortical tissue rather rapidly without conspicuous effect until a sudden collapse of the plant follows. In or on the diseased tissues, superficial, white, cottony extrametrical mycelium is usually found to be fairly abundant and young sclerotia may be present. The sclerotia start as white compact bodies of hyphae up to the size of a barley kernel, varying in this respect with the host and with environmental conditions. These normally appear on the surface of the substrate or are partially embedded and with age the outer layers of the sclerotia become jet black. In some cases, the fungus invades the pith of growing plants and without producing the usual abundant extrametrical mycelium, grows profusely and produces sclerotia within the pith cavity. This phase occurs commonly on potato, tomato, cucumber and on seed plants of lettuce, cabbage, rutabaga and turnip. In crucifer seed plants, the usual first evidence of disease is the formation of grayish linear lesions on the main stem beginning at the nodes. The final effect of pith infection is a slow stunting of the plant and premature ripening, rather than the sudden collapse, which follows concentration of the pathogen in the cortex at the base of the plant.

On mature storage organs, the same type of mycelial and sclerotial development occurs. The host tissue becomes soft and watery as the disease progresses. The decayed portion of the organ loses water, sometimes rapidly and finally desiccation follows. The effect of the host is not unlike that produced by the bacterial-soft-rot bacteria, the chief diagnostic character of watery soft rot being the conspicuous mycelium in the early stages and the sclerotia in the later stages (Walker, 1969).

5.2.7 Rapeseed-Mustard

Based on the symptoms, the disease has been named as white blight, white rot, stem blight, stalk break, stem canker and rape canker. Under natural conditions, the stem of the plant is seen affected more frequently, though all above-ground parts are subject to attack by the disease. Symptoms on the stem become visible as elongated, water-soaked lesions, which later on are covered by a cottony mycelial growth of the fungus (Plate 5.2.7.1A, B). When the stem is completely girdled by such lesions, the plant wilts and dries. Sometimes, the infection is restricted to a smaller area of pith, which results in slow stunting of the plant and premature ripening rather than the sudden collapse of the affected plants (Plate 5.2.7.1 C, D). Such plants under field conditions can be easily identified from a distance because of premature ripening (Plate 5.2.7.1E). The affected stem tends to shred and numerous grayish-white to black, spherical sclerotia appear either on the surface or in the pith of the affected stem (Plate 5.2.7.1F). When the crop is at seed maturation stage, the plants tend to lodge, touching the siliquae to the soil level. Such plants, though remaining free from stem or aerial infection throughout, show rotting of the siliquae with profuse fungal growth, along with sclerotial bodies just above the soil level. In an early stage of infection, damping-off, root rot and death of the whole plant may be



Plate 5.2.7.1 White stem rot of rapeseed-mustard. (L–R) A: Initial growth at the lower portion of the stem; B: White mycelium growth acquires more areas; C, D: Infection on the stem caused drying of the branches; E: Drying of the crop visible in the field; F: Black hard sclerotia in side the pith of the stem (Photos A, B & F. Adapted from http: //www. whitemoldresearch.com. With permission) (*See Color Plates*)

observed. Lesions on leaves are grayish, irregularly shaped and often associated with adhering petals.

Occasionally, gray mould (*Botrytis cinerea*) is associated with stem rot, covering infected areas with brown to gray fluffy mycelium.

5.2.8 Soybean

In soybean the disease is more prominent as stem rot. The symptoms of stem rot typically appear during the early stages of pod development (growth stages R3–R4). At the canopy level, foliar symptoms are the first indication that the disease is present. Foliar symptoms are chlorosis and wilt, with tissues between major leaf veins developing gray-green cast while vein tissues remain green. In time, leaves become totally necrotic, tattered and curled, but remain attached to the stem past maturity. Foliar symptoms of *Sclerotinia* stem rot could be mistaken for late season *Phytophthora* root rot, brown stem rot and stem canker, but differences in stem symptoms among these diseases can be used for diagnosis. Initially, the stem lesions develop at nodes and appear gray and water-soaked. The pathogen rapidly progresses into stem tissues above and below nodes and causes lesions that are 6–30 cm in length and usually encompass the entire stem. White fluffy mycelium covers the lesion area, especially during periods of high relative humidity (Plate 5.2.8.1B). Black sclerotia are differentiated from mycelium and are readily observed on the lesion surface. Initially lesions are tan and progressively become white, and present a sharp contrast at the interface with green stem tissues. By crop maturity, stem tissues are white and tissues have a shredded appearance if disturbed and a reddish discolouration is frequently interspersed within diseased stem tissues and at the border of lesions. At harvest, diseased stems are characterized by poor pod development, a white appearance and an abundance of sclerotia within the pith. Diseased pods are outwardly white in appearance, mycelium and sclerotia are readily observed inside and infected seeds appear white and moldy (Plate 5.2.8.1A). Sclerotia are commonly observed with the harvested grain and, if free water is present, can cause seed decay problems in storage. Most seeds harvested from affected plants are somewhat flattened and undersized being approximately quarter of the size of normal, healthy seeds. Most of these seeds appear to be normal in other respects but some show a chalky appearance with the testa wrinkled and often ruptured. Dark grey sclerotia similar to those on the pods and stems are present among the seed (Thompson and Westhuizen, 1979).

5.2.9 Sunflower

Wilt and root rot may appear at any stage of growth but becomes most conspicuous during the flowering and seed development stages. The incipient wilt is characterized by rotting through the tap root or through the hypocotyl axis. Water-soaked lesions occur on the tap root and on some fibrous roots. The above ground appearance of the lesion at the stem base is soon followed by slight leaf yellowing, quickly followed by rapid killing and desiccation of the entire plant. The lesion is continuous and extends from a tap root along the hypocotyl to as much as 50 cm above the base of the stem (Plate 5.2.9.1A). The root systems of the affected plants, particularly the fibrous roots in the upper 20 cm of soil are destroyed almost



Plate 5.2.8.1 *Sclerotinia* disease of soybean. A: Infected field showing dried plants; B: Infected stem at the basel portion of the stem (Adapted from http://www.whitemoldresearch.com. With permission) (*See Color Plates*)

completely by the time that the plants wilt and lesions appear, suggesting thereby that wilting occurs primarily due to severe root and basal rot (Plate 5.2.9.1B). The lesion referred above, is brown, wet and soft, at first it particularly surrounds the stem but it completely girdles the stem and extend upwards. On such lesions on the stem and on the stem below the soil level, dense, white growth of the fungus

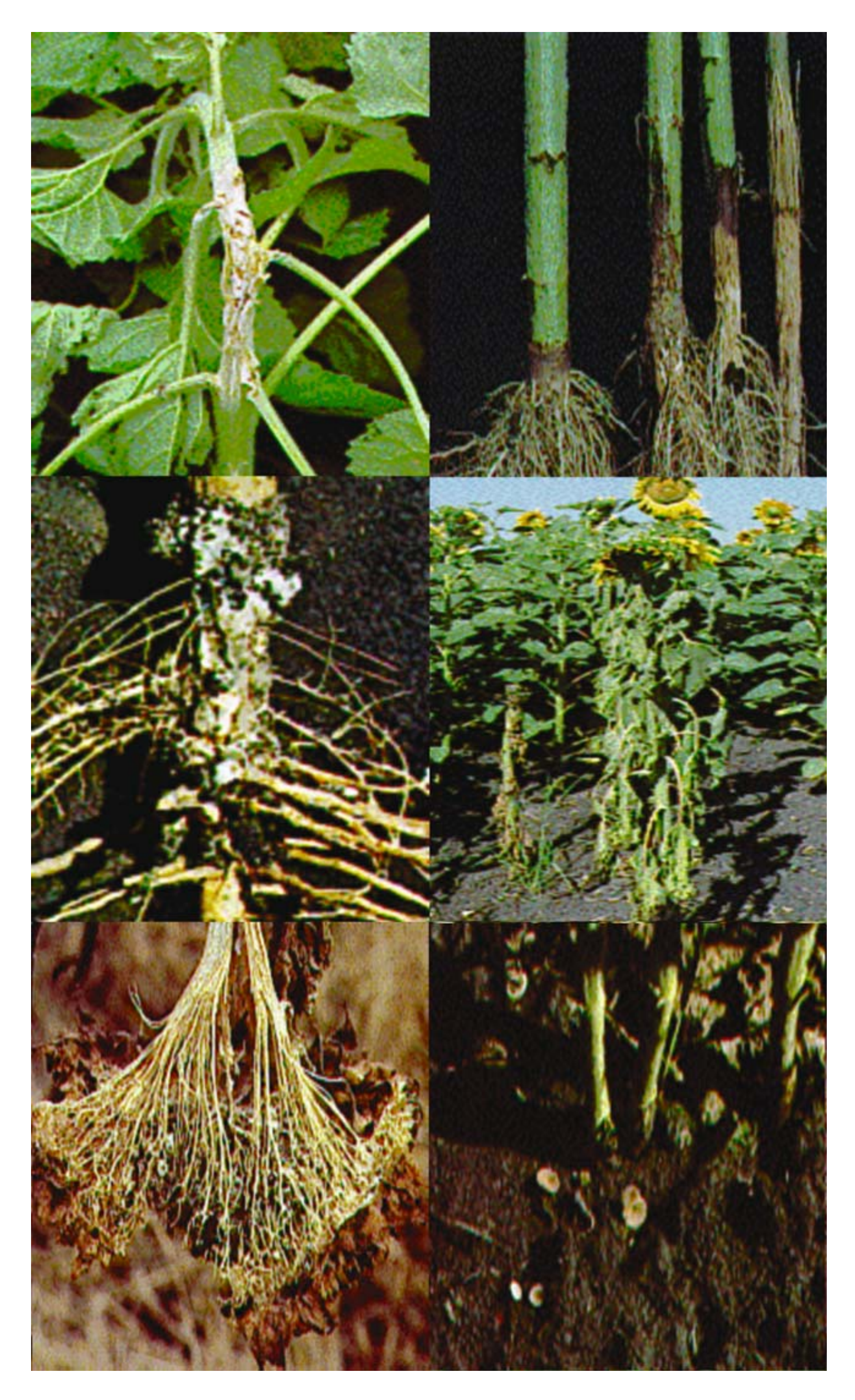


Plate 5.2.9.1 *Sclerotinia* disease of sunflower. (L–R) A: Mycelium growth at the middle of the stem; B: Basel canker formation at the base of the stem; C: White mould near the soil level; D: Sudden wilting of the plants in the field; E: Head rot due to *Sclerotinia* F: Apothecia formation on the soil (Adapted from http://www.whitemoldresearch.com. With permission) (*See Color Plates*)

can be seen with loosely attached black sclerotial bodies which are irregular in size and shape. Similar bodies are found in stem and root piths (Plate 5.2.9.1C). The earlier a plant is infected, the more severe the symptoms and the fewer seeds develop. Sudden "drop" when all leaves of a plant wilt within a few days are a characteristic severe symptom of the disease (Plate 5.2.9.1D). Stems of severely diseased plants become shredded into dry, straw-coloured fibers. The diseased plants occur scattered in the rows often singly at first and soon in groups of two or more plants, until near harvest when continuous portions of rows are diseased. Spread of the disease from one plant to another appears to take place by root contact. In warm weather, the disease develops rapidly, plants appearing healthy today may be wilted completely on the next day.

Stem or stalk rot symptoms appear later in the season where such infected plants may not wilt and the only exterior symptoms may be a small brown lesion at the stem base or at any part of the stem, often in the upper half. Stems of severely diseased plants shred into vascular strands, becoming straw-coloured as they dry. Such stems are weak and the plants lodge easily. Symptoms of stem rot in the upper half of the stem are usually due to air-borne ascospores formed in the apothecia (Plate 5.2.9.1F). The rotted part of the stem may or may not show the presence of sclerotia.

Head rot infection is by air-borne ascospores and is dependant on heavy rains occurring during flowering and seed development stages. The symptoms may not become visible in any part of the receptacle. Rotting usually starts as a water-soaked, light brown wedge at the outside of the green head. Aided by cool temperatures, the rot spreads and results in the partial or complete destruction of the head, including the seed. White mycelium is usually abundant and is converted into sclerotia. Rotted tissue, seed and sclerotia fall to the ground and in severe cases only a straw-coloured, broom-like remnant is left. Severely affected heads show shredding of the tissue, resulting in incomplete filling of the head with seed (Plate 5.2.9.1E). The head rot may be partial or complete. Seeds formed on partially affected heads may show the presence of sclerotia on their surfaces.

5.2.10 *Safflower*

Infected plants become yellowish, turn brown, wilt and die. Large black sclerotia are formed on the crown, inside the stem and on adjoining roots, though the roots themselves are not generally affected. Shredding of the cortical tissues of the lower stem takes place. At an advanced stage of the disease, flower heads can fall from the affected plants, leaving the outer involucral bracts in situ. A brown discolouration and rotting at the base of the capsules is common. This effect is considered to be caused by the development of large-sized sclerotia in the thalamus of the capitulum. Plants only slightly affected do not show major symptoms, but at maturity heads often contain few or no seeds. The presence of white mycelium and black sclerotia on or around the affected plants is also seen. The severity of the disease increases with the increase in rainfall.

5.2.11 Peanut

In peanut usually the pegs are invaded first at the soil level, facilitating the colonization of the lateral branches. Light-tan to brown lesions demarcating healthy and affected tissues appears on the lateral branches. The lesions then become dark brown, shredding of the tissue becomes evident. From an affected branch, the fungus moves into and colonizes the main branch also. Leaves of such plants become chlorotic, turn brown and wither, resulting in defoliation and death of the lateral branches or the whole plant. Pods of severely affected plants also show rotting. The tap root becomes necrotic and turns black in colour. Abundant white fluffy mycelium appears on the soil surface in close proximity of affected parts or debris in the field. Sclerotia of the fungus are also produced on the surface and within the affected branches, in the central portion of the tap root, on the pegs, on the surface of the pods, on the interface of the shell and inside on the seed.

5.2.12 Beans

The white mould of beans affects stems, leaves and pods infect all parts above ground and to a certain extent below ground. The first symptoms appear as soft, watery, irregular spots which under cool, moist conditions enlarge rapidly. These spots soon become covered with an extensive white growth of the fungus. Under certain conditions of heavy soils, plants appear light yellow, gradually become darker, and may die prematurely. Often main stems and branches become girdled, from which they wilt and die (Plate 5.2.12.1A). The pods, particularly those in contact with the soil, are quickly infected and soon become a watery mass upon which appears a heavy cover of white mycelium or thread-like growth (Plate 5.2.12.1B). These dense masses of mould have the appearance of small bits of snow. Soon they turn grey and gradually dry out, becoming hard, black bodies known as sclerotia. The tissues of affected plants become dry, bleached, and pinky in nature (Plate 5.2.12.1C). Bean seeds attacked by the fungus appear dull and chalky and are usually lighter in weight than are healthy seeds (Starr et al., 1953). According to Steadman (1983), the disease first appears as wilted leaves scattered in a field. When the vines are observed more closely, soft, watery spots on leaves, pods or stems can be seen. Each lesion enlarges to become a rotted, watery piece of tissue covered with the white mycelial signs of the fungus. When stems or branches are attacked, wilting occurs and branches eventually die and take on a dry, bleached appearance. The sclerotia form in and on affected plant parts. The bleached stem symptom and sclerotia formation are diagnostic of white mould. Normal senescence or stress from drought or other bean diseases causes the plant to turn yellow to tan as it dries without associated sclerotia.

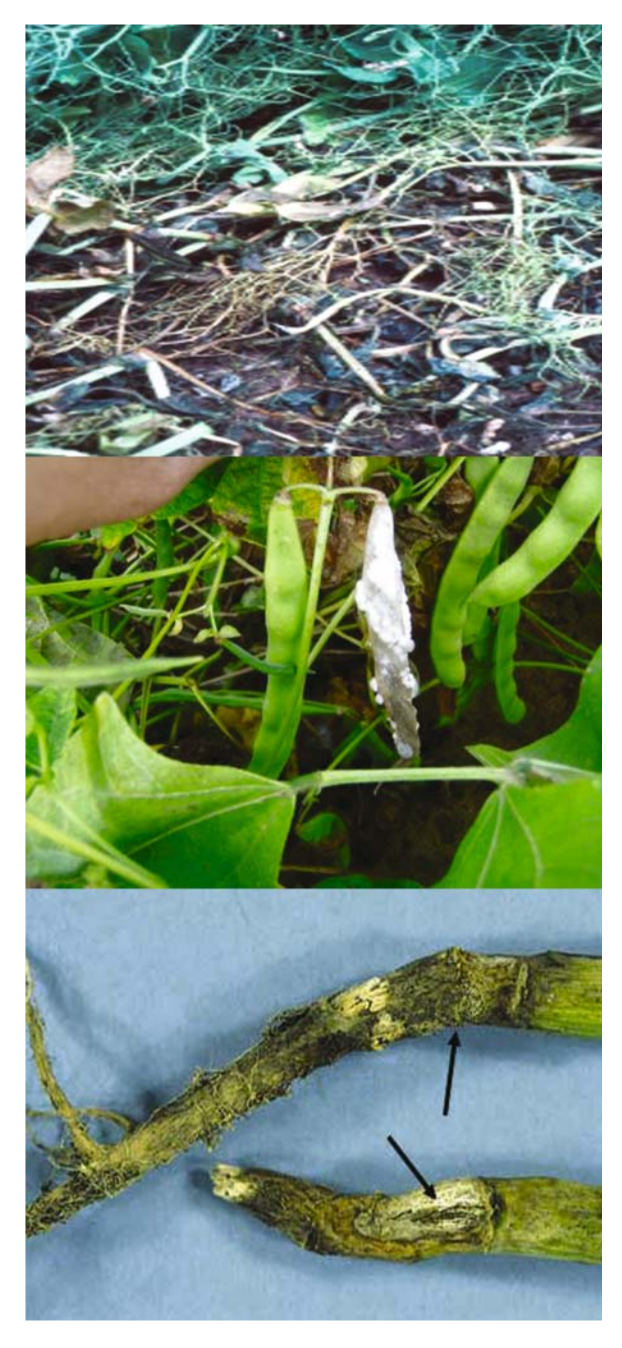


Plate 5.2.12.1 *Sclerotinia* disease of pea/beans. A: White mold infection on peas; B: White mold infection on pods; C: *Sclerotinia* infection at basel stem portion (Adapted from http: //www. white- moldresearch.com. With permission) (*See Color Plates*)

5.2.13 Carrot

In carrot, the disease is known by the name of cottony soft rot or *Sclerotinia* soft rot. The disease was first reported in carrots in Belgium in 1860. Lesions caused by *Sclerotinia* always exhibit characteristic cottony white mycelium on the surface of infected areas. When first formed, these lesions may be confused with those caused by *Rhizoctonia carotae*, however, the latter are sunken and more firm. Sclerotia on the surface of lesions confirm the presence of *Sclerotinia*. The decay typically is a soft, watery rot. It can be distinguished from bacterial soft rot by the absence of sliminess. Secondary organisms frequently gain entrance into the *Sclerotinia* areas and quickly change the tissues into a soft, mushy, malodorous mass (Sherf and Macnab, 1986). In Finland, carrots with unusual cavities on tap roots after one month of storage in plastic bags have been observed due to *S. sclerotiorum* (Koponen and Valkonen, 1996).

Symptoms on foliage first appear as water soaked dark olive green lesions associated with collapsed tissue (Plate 5.2.13.1A). Lesions expand rapidly over the entire leaf, petiole and rosette with infected tissues soon becoming covered by abundant cottony white mycelium (Plate 5.2.13.1B). Aerial hyphae usually appear about 10 mm behind the advancing discolored lesions (Geary, 1978). At an advanced stage of disease progress, affected tissue exhibits a bleached appearance and occasionally an entire plant may collapse (Plate 5.2.13.1C). Eventually large black sclerotia (2–20 mm) form externally (Plate 5.2.13.1D) embedded in the mycelium or internally within the path of the petiole. Typically lesions on stored roots that are infected from the field develop in the crown region as localized softened tissue and white mycelium tufts erupting through the cuticle (Plate 5.2.13.1F). Mycelium from a single infected carrot can spread rapidly to adjacent roots forming radiating pockets on infection (Plate 5.2.13.1E) with ramifying hyphae reaching up to 25 cm from the source. Lesions caused by this secondary spread of the pathogen can occur anywhere on the root and initially appear as water soaked circular spots characterized by a slight discolouration of the infected tissue. At a later stage, the expanding lesions develop into soft, watery, odourless rot, characterized by darking of the invaded tissue and the presence of a rapidly spreading white mycelium. Colonized carrots are usually held together in large clumps by the extensive mycelial growth. The formation of individual large sclerotia on infected areas is a distinctive features that differentiate *Sclerotinia* rot of carrot from other storage rots such as gray mold rot (*Botrytis cinerea* Pers. Fr.), crater rot (*Rhizoctonia carotae* Rader) and bacterial soft rot (*Erwinia* spp.). In addition, secondary organisms may gain entrance into infected areas and contribute to further disintegration of macerated tissue and the complete collapse of infected roots.

5.2.14 Celery

Celery pink rot has been reported since the early 1920s in most celery growing areas of the USA. Celery is affected at all stages of growth and storage. Damping-off occurs in seedbeds. Seedling symptoms usually appear first on the stem near the



Plate 5.2.13.1 *Sclerotinia* disease of carrot. A. Leaves and petioles showing symptoms of *Sclerotinia*; B: Lesions advancing on carrot petioles; C: Collapsed leaves and petioles due to severe field infection; D: Sclerotia production on diseased leaves and soil surface; E: Secondary spread of foliar infection in the field; F: Mycelium of *S. sclerotiorum* erupting from the crown of a stored carrot originally infected in the field (Adapted from the publication of Kora et al., 2003. With permission) (*See Color Plates*)

soil surface. Here a watery soft rot develops, later the tops fall over and die and the fungus spreads to adjacent plants. White, cottony mycelium is abundant on affected plant surfaces during humid conditions. *Sclerotinia* rot on celery in production fields and in storage is called as "pink rot" and "water soft-rot". The base of the stalks is affected first and may turn pinkish or reddish brown, especially at lesion margins. Entire stalks finally rot and become brownish, watery, and covered by the cottony white fungal growth. The rotted tissue is odourless unless other organisms

follow and further decompose the tissue. Hard black sclerotia, about 1/8 to 1/4 in. in diameter, frequently forms on or within the white mould. Sclerotia produced at temperatures near the minimum for fungus growth tend to be larger than those produced near the optimum temperature (Sherf and Macnab, 1986). Petiole and crown rot symptoms are common in California (Koike et al., 2006).

5.2.15 Lettuce

This serious disease known as “drop” or *Sclerotinia* rot is in the USA since 1894. The name “drop” best describes the final symptom on lettuce. The rot usually begins on the stem near the soil surface and a water-soaked area appears. It can spread downward until roots are decayed and can spread upward until leaf bases are affected. Petiole rot causes leaves to die, wither and droop until their tips rest on the ground. The pathogen rapidly ascends the stalk, killing the leaves in succession until it reaches the heart of the lettuce plant. Each leaf in turn drops downward until it rests on the one below. Inner leaves do not dry out as quickly as outer leaves and therefore are invaded completely by the fungus, which reduces the head to a wet, slimy mass. Under moist conditions, the fungus produces a snowy white weft over the entire head. In this weft, especially among the collapsed leaves, numerous, black, variously shaped but not flat sclerotia develop. These may be as small as mustard seed or as large as a bean, depending on the fungus species and the temperature during their formation. Presences of the white mycelial mass and black sclerotia are signs that differentiate drop from other head rots (Sherf and Macnab, 1986).

5.2.16 Linseed

The pathogen *Sclerotinia fuckeliana* occurs both as a parasite and a saprophyte on host plants and causes ‘grey mould’ or ‘botrytis disease’, a blight or rot of immature, fleshy or senescent tissues. The pathogen first cause damping-off and basal leaf and stem rot when crop is small. Later on lesions develop on the stem as tan or brown water soaked areas, which may become greyish on drying out. The profuse grey brown sporulation of the fungus on old diseased tissue is a characteristic features. Rotting of plant produce at harvest or in store causes large losses. Blights of buds, blossom, leaves and stems may also occur and may result into dieback. Some time canker formation also takes place on woody plant parts. Conidia are formed in humid temperate or sub-tropical areas become air-borne, but may be carried on the surface by rain splashes. Diseased plant parts, on which sporulation is profusely in wet weather, are important sources of inoculum in disease epidemics. The fungus over winters as sclerotia or as mycelium in old plant debris and may be seed borne as spores or mycelium.

5.2.17 Potato

Sclerotinia stem rot first appears as water soaked spots, usually at the point where stems attach to branches or on branches or stems in contact with the soil. A white cottony growth of fungal mycelium develops on the lesions and infected tissues become soft and watery (Plate 5.2.17.1A, B). The fungus may spread rapidly to nearby stems and leaves if moisture is present for several hours. Lesions then may expand and girdle the stem, causing the foliage to wilt (Plate 5.2.17.1C, D). During dry conditions, lesions become dry and will turn beige, tan or bleached white in color and papery in appearance. Hard, irregularly shaped resting bodies of the fungus called sclerotia, form in and on decaying plant tissues. Sclerotia are generally 1/4 to 1/2 in. in diameter, initially white to cream in colour but become black with age (Plate 5.2.17.1E). These sclerotia frequently develop in hollowed out centers of infected stems, which eventually fall to the ground where the fungus is able to survive until the next growing season.

5.2.18 Opium Poppy

Initial symptoms appear on the stem near the collar zone as water soaked lesions (Plate 5.2.18.1A) and later in the form of white cottony growth which starts rotting. After some time, irregular black coloured sclerotia appear on the infected stem and capsule (Plate 5.2.18.1B). In severe conditions, abundant sclerotia are produced on flower bud (Plate 5.2.18.1C) and capsule (Plate 5.2.18.1D). The severely affected capsules produce no seeds as they transform into sclerotia of 5–10 mm in diameter (Plate 5.2.18.1E, F).

5.2.19 Lentil

Sclerotinia stem blight or White mold of lentil occurs in the field from early flowering to pod setting, usually in highly productive fields with tall, dense stands of lentils. Lentil plants infected by pathogen first appear bleached near infection site on stems, leaves and stems turn brown to tan and die prematurely (Plate 5.2.19.1A). Infected areas are covered with white fluffy mold growth of the pathogen (Plate 5.2.19.1B). Dark brown to black sclerotia develop inside and often outside of the infected plants which later produce apothecia at the infected plant or at soil level (Plate 5.2.19.1C).

The disease is favoured by wet and cool conditions especially on lower ground where dense canopies usually develop. Because winter lentils are exposed to the prolong wet and cool spring weather. Disease is likely to be more common and more severe in winter lentils than in spring sown crop.



Plate 5.2.17.1 *Sclerotinia* stem rot of potato. A, B: *Sclerotinia* infection at the base; C: *Sclerotinia* causing drying of the stem; D: Drying and breaking of the stem; E: Breaking and production of black sclerotia (Adapted from http://www.potatodiseases.org. With permission) (*See Color Plates*)



Plate 5.2.18.1 *Sclerotinia* disease of poppy; A: Basal rot of poppy; Abundant apothecial production under field conditions: B: Healthy (left) and infected (right) stem and mummified capsule of poppy; C: Flower buds heavily infected with pathogen showing white colony growth intermingled with sclerotia; D: Capsule of poppy showing sclerotia in side. E: Capsule of opium poppy showing infection of *Sclerotinia*, Black sclerotia on capsule; F: L.S. of infected (left) capsule showing fungal growth and sclerotia with healthy capsule (right) (Adapted from the publication of Singh and Singh, 2003. With permission) (*See Color Plates*)

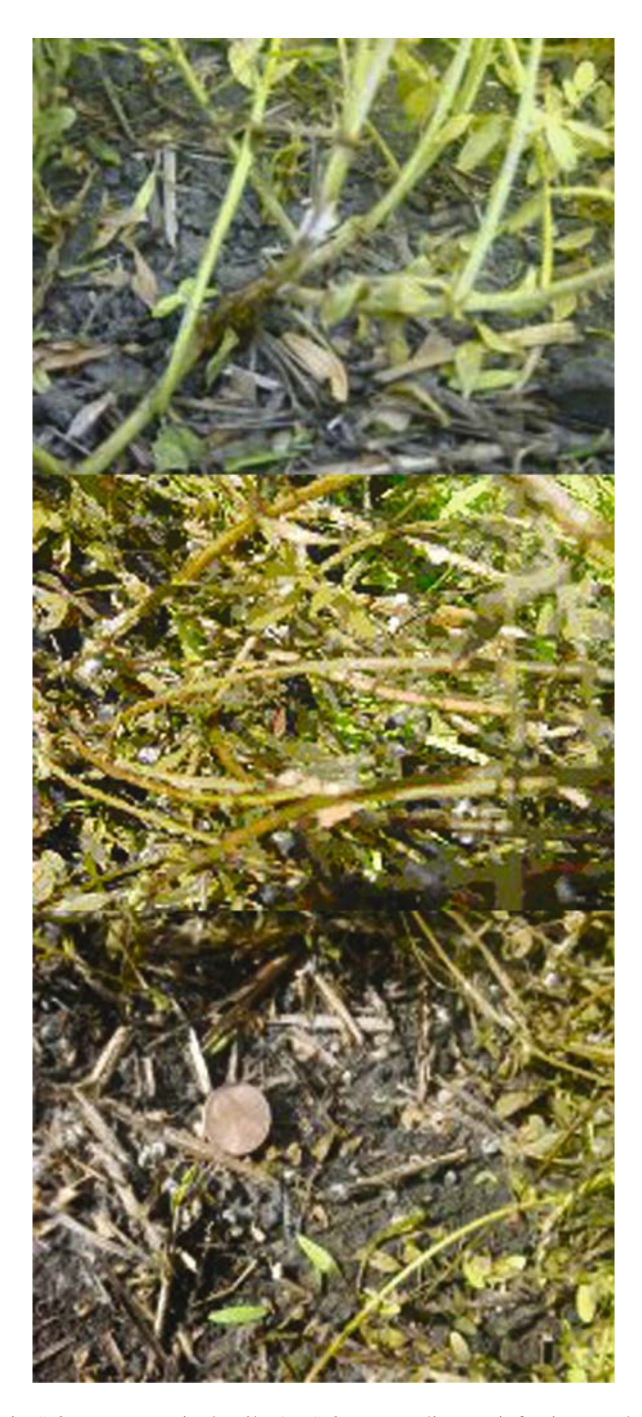


Plate 5.2.19.1 *Sclerotinia* rot in lentil. A: *Sclerotinia* disease infection on lentil stem; B: Severe infection at the base; C: Apothecia production at the soil level (Adapted from http:// www. whitemoldresearch.com. With permission) (*See Color Plates*)

5.2.20 *Buckwheat*

The disease is characterized by the appearance of water-soaked areas on the upper portion of the root, which gradually proceeds downwards covering the whole root system.

The water-soaked lesions later turns into brown patches. The above ground foliage wilts and quickly dies. The disease spread rapidly with cloudy, humid weather and day temperature ranging from 15–20°C (Mondal et al., 2003).

5.2.21 *Mungbean and Urdbean*

The disease is characterized by blighting of stem, leaves, branches, flower stalks and pods at varying intensities during wet period. The presence of dark sclerotia of various sizes on the surface (0.2 × 0.2 – 0.8 × 0.3 cm) and pith (0.6 × 0.4 – 5.7 × 0.1 cm) of infected plant parts is evident (Bag, 2003b).

5.2.22 *Cucumber*

The disease initiates as water soaking followed by a white mycelial growth covered in dew-like drops of water on infected cucumber fruits. Three to five days later, the fruits completely rot. Similar symptoms appear on leaves, stem and petioles. Stem cankers covered with thick cottony white mycelium are observed causing water soaking on petioles, leaves and buds resulting in necrotic spots on leaves (Zakeri, 1998).

5.2.23 *Pepper*

In this case, infected plants foliage become pale green initially and later show wilting. Crowns develop brown lesions that girdle the plant resulting in plant death. White mycelia and small (2–3 mm), black, irregularly shaped sclerotia are observed on the outside of plant crowns and in the centre of stem cavities (Gonzalez et al., 1998).

5.2.24 *Chickpea*

Initial symptoms include wilting of leaves and stem necrosis on individual branches followed by entire plant necrosis and death (Plate 5.2.24.1A, B). White mycelium



Plate 5.2.24.1 *Sclerotinia* rot of chickpea. A: Drying of leaves at the initial infection; B: Severe infection cause drying of the stem; C: Mycelium and sclerotia formation at soil level; D: Sclerotia sticking to stem (Adapted from http:// www. whitemoldresearch.com. With permission) (*See Color Plates*)

is present on plant stems near soil surface (Plate 5.2.24.1C). Small black irregularly shaped sclerotia (1 mm in diameter) are present on the infected stem surface along with the white mycelia (Plate 5.2.24.1D). Sometimes larger sclerotia with 5–6 mm in diameter are also common (Matheron and Porchas, 2000).

5.2.25 Dollar Spot of Turf Grass

The disease name is derived from the dead straw-colored spots about the size of a silver dollar on close cut bent grass putting greens. Dollar spot may persist from early summer until early fall and its incidence seems to be higher in seasons with low rainfall, presumably from the adverse effect of low soil moisture on host plants. It occurs on bluegrasses, bent grasses, fescues, and zoysia. The classification of the pathogen that causes dollar spot has under gone numerous changes. Previously it was *Sclerotinia homoeocarpa*, now it is considered, *Lanzea* sp. and *Moellerodiscus* sp. The symptoms attributed to “Dollar spot” probably are caused by more than one species.

5.2.25.1 Symptoms

The pattern of symptoms depends largely on mowing practices. Under close mowing conditions, the circular straw-colored spots (3–6 cm in diameter) are distinctly outlined in the early stages of disease development (Plate 5.2.25.1D, E). With higher cutting heights, the bleached turf spots are irregularly shaped. In the early morning, when dew is still on the grass, a white cobweb by growth of the fungus may be seen over the spot (Plate 5.2.25.1C). Spots coalesce to cover large areas when the disease becomes severe. On individual grass blades the damaged tissues are first water soaked and dark coloured. As they dry, the lesions turn light tan to straw-coloured with a reddish-brown border (Plate 5.2.25.1B). The lesions first occur randomly on the leaf blade and then frequently extend across the entire blade (Plate 5.2.25.1A).

5.2.25.2 Disease Cycle

The fungus, *Sclerotinia homoeocarpa*, survives unfavorable periods as dormant mycelium in infected plants, therefore, fungal movement is brought about by equipment, people, animals, wind or water. When day time temperatures reach 16–27°C, the dormant mycelium resumes growth from the infected leaves to nearby healthy leaves, causing new infections. If night-time conditions become cool and dry soon after infection has occurred, infection may not progress beyond scattered leaf lesions. If the grass is growing rapidly, the problems may disappear after one or two mowings. If favourable weather persists after infection such as warm nights, with dew forming on leaves, entire grass plants may be killed and typical "dollar spots" may appear on the turf.

5.2.25.3 Management

Mow grasses at the recommended maximum height if possible. Try not to remove more than 1/3 of the leaf surface in any one mowing. Maintain adequate soil moisture, but avoid sprinkling in the late afternoon or evening. Do not over water. The incidence of dollar spot is lower on nitrogen deficient turf. Adequate nitrogen fertilization in the late spring and summer may help prevent dollar spot, but excess applications may encourage other turf problems (e.g., brown patch, summer patch and drought stress). Varieties of bluegrasses and fescues differ in susceptibility to dollar spot. Blue grasses which exhibit greater resistance include the improved varieties Adelphi, America, Aquila, Bonnie blue, Bristol, Eclipse, Midnight, Touchdown, Vantage and Victa. Greater susceptibility is exhibited by varieties which include Ram I, Mystic, Estar, Gnome, and Pennstar. Fescues which are more resistant include Jamestown, Agram, Checker, and Shadow chewings, Biljart, Reliant, Scaldis and Tournament hard fescues. Many fungicides are available for dollar spot management. For home owners use systemic fungicides containing the



Plate 5.2.25.1 Dollar spot of turfgrass. A: Dollar spot initiation on bent grass; B: Tan shaped lesions on the bent grass; C: Infected area on the leaf blade; D: Dense white mycelium on seedling turf; E: Large masses of hyphae/ mycelium on the lawns (Adapted from http://www.turf – grass management.psu.edu; http://www.caes.uga.edu; http://www.ces. ncsu.edu. With permission) (*See Color Plates*).

active ingredients: thiophanate-methyl, triadimefon, propiconazole or myclobutanil or protectant fungicides containing chlorothalonil, mancozeb, quintozene or thiram. Systemic should be drenched into the turf for longer lasting control.

5.2.26 Clover

The disease is known as crown or stem rot. However, it is known by various other names like clover sickness, clover rot, stem rot or merely rot. In India, the disease generally appears in the last week of December and become severe during January and February. In western countries, it appears at the time of fall. The infection originates from the ascospores from the apothecia produced in the months of August to October. The symptoms first appear in the fall as small, brown spots on leaves and petioles. The heavily infected leaves become grayish brown, wither and become over run with white mycelium which spreads to the crown and roots. Later on crown and basel parts of young stem shows brown soft rot which extends downward to the root. In Indian conditions, generally the infection starts as brown lesions on the collar region of the stem. The infected leaves become yellow, die and turn brown. Leaves fall to the soil surface and from them a white mycelium growth develops that infects stems and from stems progressively decay and watery soft rot develops. White mycelium may develop on stem, leaves and on soil where these plants lay and form sclerotia in the mycelial mass. The roots near the soil surface may get affected.

Under humid conditions, some of the masses of mycelium changes into small hard, black cartilaginous bodies (sclerotia) in the late winter. They are attached to the soil surface of the dead stem, crowns and roots or in the soil near the roots.

5.2.27 Alfalfa or Lucerne

The disease is also known as crown or stem rot. The disease causes patchy stands loss in the spring of the year. Primarily it attacks late summer and fall planted alfalfa. The disease attack in the fall and grows throughout the winter especially in the areas with moderate winters and heavy snow fall. Fall seeded alfalfa can be infected during the seedling stage. The infected plant stems and leaves wilt and turn yellow to grayish green. These symptoms are generally not noticed since these are associated with those caused by frost damage. The infected plant die as the infection progress and later on hard black sclerotia form on the root just below the soil surface. In the severe cases, disease is visible as dead alfalfa leaf litter on the soil surface.

The infection can occurs on established plants but symptoms are like newly planted stems. The leaves and stems of the infected plants turn yellow or grayish green and then collapse. Some times affected plant may have few green shoots remaining even though the other shoots have died back. Sclerotia usually appear in the crown areas or inside the dead stems. In the severe cases, the plant eventually dies.

Chapter 6
Disease Assessment

A number of disease assessment scales have been used by different workers for different crops as follows:

6.1 Beans

A 0–5 disease severity scale was used by Abawi et al. (1978) to evaluate bean germplasm for resistance to *Sclerotinia*.

0 = No apparent symptoms,
1 = 1–3 arrested, small leaves,
2 = One to several running lesions with moderate mycelial growth,
3 = Mycelial development involving up to 25 per cent of foliage,
4 = Extensive mycelial growth covering up to 50 per cent of the foliage and
5 = Death of the plants caused by massive mycelial growth.

Plants scoring 0–1 are considered resistant and 2–5 as susceptible.
Huang et al. (1988) assessed white mould of dry bean incidence by dividing into five categories based on percent infected plants in each field.

1 = No disease,
2 = Trace, <1 per cent,
3 = Light, 1–10 per cent,
4 = Moderate, 11–25 per cent and
5 = Severe, >50 per cent.

Godoy et al. (1990) used 1–7 scale for stem and 1–5 for leaf infections in *Phaseolus vulgaris*.
Stem Infections

1 = Detectable symptoms to tiny necrotic specks,
2 = Lesions up to 1/6th diameter of stem,
3 = Lesion size from 1/6th up to 1/2 diameter of stem,
4 = Lesion up to 30 mm long,

G.S. Saharan, Naresh Mehta, *Sclerotinia Diseases of Crop Plants: Biology, Ecology and Disease Management*,

5 = Lesion up to 30–50 mm long,
6 = Large lesion, stem girdled but plant not showing wilt or stress and
7 = Large, discoloured, dry or soft lesion and stem bent.

Leaf Infections

1 = No detectable lesion,
2 = Lesion diameter <1.00 cm,
3 = Lesion diameter between 1.0 and 2.0 cm,
4 = Lesion diameter between 2.0 and 3.0 cm and
5 = Lesion diameter >3.0 cm.

Morton and Hall (1989) estimated disease severity of white beans on a scale of 0 to 4 where:

0 = 0 per cent,
1 = 1–25 per cent,
2 = 26–50 per cent,
3 = 51–75 per cent and
4 = 76–100 per cent of the surface area of the shoot with symptoms of white mould.

6.2 Soybean

To assess soybean germplasm resistance, Grau et al. (1982) used 0–3 scale:

0 = No symptoms,
1 = Only lateral branches showing lesions,
2 = Lesions on the main stem, but little or no effect on the pod fill and
3 = Lesion on main stem resulting in plant death and poor pod fill.

$$\mathrm{DSI} = \frac{\text{Class} \times \text{No. of plants in class} \times 100}{\text{Total number of plants} \times 3}$$

Cline and Jacobsen (1983) suggested 0 to 5 rating scale for evaluating soybean cultures.

0 = No symptoms,
1 = Water-soaking of flowers and/or arrested, small lesions in the axils or on the main stem,
2 = Water-soaking of petioles and leaves only and/or leaf drop,
3 = Lesions on the main stem resulting in stem collapse and/or mycelial growth covering up to 25 per cent of foliage,
4 = Mycelial growth covering up to 50 per cent of foliage and
5 = Dead plant.

Chun et al. (1987) used 0–3 scale.

0 = No disease,

1 = Localized lesions,
2 = Expanded lesions but mostly not affecting pod fill and
3 = Expanding lesions resulting in poor pod fill or plant death.

6.3 Sunflower

Sunflower germplasm against wilt has been assessed by Huang and Dorrell (1978) on a 1–4 scale.

1 = Resistant, no wilting,
2 = Moderately resistant, wilting of tips of the first pair of true leaves,
3 = Susceptible, wilting of the entire area of the first pair of true leaves and
4 = Highly susceptible, wilting of all leaves.

A wilt index (W) is calculated for each line using the formula W = (nW)/T, where
n = Number of seedlings,
W = Wilt rating and
T = Total number of seedlings.

To assess head rot intensity of sunflower Hampel et al. (1981) used 1–4 scale.

1 = No disease,
2 = <10 per cent disease,
3 = 10–25 per cent disease and
4 = >25 per cent disease.

6.4 Peas

Resistance in field peas germplasm has been assessed by using a 1–6 rating scale Blanchette and Auld (1978).

1 = No symptom,
2 = Lesion less than 1.0 cm in length,
3 = Lesion 1.0–2.0 cm in length,
4 = Lesion 2.1–3.0 cm in length,
5 = Lesion 3.1– 4.0 cm in length and
6 = Lesion greater than 4.1 cm in length.

6.5 Clover

A visual assessment key was developed by Dixon and Doodson (1974) to test resistance of red clover to *Sclerotinia* rot. Photographs of infected plants are collected from plots in a number of trials. These are divided into four categories:

0 = Healthy,
1 = Slight symptoms,
2 = Moderate symptoms and
3 = Severe symptoms.

A representative photograph is selected from each category and treated over with Indian ink. The photograph is reduced leaving only the ink-out line which is re-photographed to give the key illustrated in Plate 6.5.1. Subsequently for field assessment 50 single plant samples are categorized according to the key. The data obtained by this method is converted into a disease index as follows:

$$DI = \frac{100[(1\times X)+(2\times Y)+(3\times Z)]}{n}$$

Where n = total number of plants assessed X, Y, Z = numbers in each category.



Plate 6.5.1 Disease Intensity key for the clover (Adapted from the publication of Dixon and Doodson, 1974. With permission)

6.6 Rapeseed-Mustard

Stem rot of oilseed rape is assessed on a 0–4 scale (Sansford, 1995) as follows:

0 = No visible lesion,
1 = 0.1–2 cm lesion length on stem,
2 = 2.1–4 cm lesion length on stem,
3 = 4.1–6 cm lesion length on stem and
4 = >6 cm lesion length on stem as complete dried plant.

Chapter 7
The Pathogen – *Sclerotinia*

Despite the continued interest of mycologists and plant pathologists, the taxonomic position of the economically important, plant pathogenic species of *Sclerotinia* has remained unresolved. Over 250 species of diverse relationships, both pathogenic and non-pathogenic, have been assigned to the genus *Sclerotinia* with consequent controversy and confusion over generic limits (Kohn, 1979a). Attempts to delimit the pathogenic species assigned to the genus using a few traditional characters have resulted in recognition of too many or too few species in relation to what is now known about the biology and micro-anatomy of this group to satisfy the practical need of the plant pathologist to name the pathogen in hand. Examination of type specimens of preserved material as well as observation of living isolates in the light of micro-anatomical and cultural characters employed by contemporary discomycotina taxonomists has resulted in the delimitation of plant pathogenic species of *Sclerotinia* in the revised and more limited circumscription of the genus presented.

7.1 Taxonomy and Nomenclature

The family Sclerotiniaceae was erected in 1945 by Whetzel (1945) to accommodate inoperculate discomycetes that produce stromata, stipital apothecia, ellipsoidal ascospores and globose spermatia. The keys and diagnoses to the genera assigned to the Sclerotiniaceae, the stroma type, ascospore colour, the presence of functional conidial state and type of conidia as major characters in delimiting new and revised genera has been provided. The genus *Sclerotinia* was designated type genus of the Sclerotiniaceae.

Whetzel recognized two basic types of stroma: one is the substratal stroma, an indeterminate type of stroma with a medulla consisting of a portion of the host substrate permeated by hyphae and with a thin black rind covering at least a portion of the stromatal surface. Genera placed in the Sclerotiniaceae by Whetzel and subsequent authors with this type of stroma included *Lambertella*, *Ciboria*, *Ciboriopsis* [=*Moellerodiscus*], *Lanzia*, *Poculum*, *Rutstroemia* and several apparently unnamed genera. The other type of stroma, a distinct sclerotium, either may develop within host tissues with remnants of these tissues remaining within the sclerotial medulla,

G.S. Saharan, Naresh Mehta, *Sclerotinia Diseases of Crop Plants: Biology, Ecology and Disease Management*,

as in *Ciborinia*, *Verpatinia*, *Monilinia*, *Myriosclerotinia*, *Phaeosclerotinia*, *Scleromitrula*, *Seaverinia*, *Botryotinia* and *Streptotinia* or may develop free from the tissues of the suspect as in *Sclerotinia* and *Martininia*, in which suscept tissues are not embedded in the sclerotial medulla. In *Stromatina*, two types of sclerotium are formed; a thin, effuse mantling stroma (with medulla and rind) and small, black "sclerotules" produced above the mantling stroma on aerial mycelium. A simple laboratory technique for determining whether suscept tissues are incorporated in the sclerotial medulla has been reported by Noviello and Korf (1961).

Another valuable character used by Whetzel and subsequent authors is the presence of a functional conidial state. Sclerotium forming genera with known anamorphic (conidial) state include *Phaeosclerotinia* (*Monilia*), *Monilinia* (*Monilia*), *Pycnopeziza* (*Acarosporium*), *Scleromitrula* (unnammed), *Ovulina* (*Ovulitis*), *Botryotinia* (*Botrytis and Amphobotrys*), *Streptotinia* (*Streptobotrys*), *Seaverinia* (*Verrucobotrys*), *Gloeotinia* (unnamed) and *Septotinia* (*Septotis*). The remaining genera, including *Sclerotinia* have no known functional conidial state. Within the Sclerotiniaceae, only three genera produce brown ascospores: *Lambertella*, *Martininia*, and *Phaeosclerotinia*, the remaining described genera produce hyaline ascospores. Although virtually ignored by Whetzel, characters of the sterile tissues of the apothecium and sclerotium have been considered by Buchwald (1949), Dumont (1971) and Korf (1973) in delimitation of genera within the Sclerotiniaceae. In addition to the development of a free, discrete sclerotium of a functional conidial state and production of hyaline ascospores (Kohn, 1979a) delimited the genus *Sclerotinia* in even more restricted sense to include only those species in which the ectal excipulum, or outer most layer of the apothecium is composed of globose cells in chains oriented perpendicularly to the receptacle surface. *Myriosclerotinia*, a genus segregated from *Sclerotinia* produces sclerotia within the culms of sedges, rushes and grasses and produces a Myrioconium state (probably spermatia) in locules within host tissues. In contrast, superficial development of both the sclerotia and the *Myrioconium* state occurs in *Sclerotinia* sensu Kohn (1979a). The two genera also show anatomical differences in tissue structure of the apothecium.

7.2 The Correct Name for *Sclerotinia*

Although Kohn (1979a) accepted *Sclerotinia* in the restricted sense but many workers, including Dennis (1978) circumscribed *Sclerotinia* in the broad sense to include *Monilinia*, *Ovulinia*, *Ciborinia*, *Botryotinia*, *Myriosclerotinia*, and *Sclerotinia*. This is a taxonomic decision and therefore, opens to opinion. The genus *Sclerotinia* was erected by Fuckel (1870) to accommodate *Sclerotinia*, *Candolleana*, *S. fuckeliana*, *S. libertiana* (an obligate synonym of *S. sclerotiorum* erected by Fuckel to avoid a supposed autonym), *S. tuberosa*, and *S. baccata*. Of these original species, *S. baccata* has been transferred to the Pezizales and is of no further interest in relation to *Sclerotinia*. Whetzel (1945) transferred *S. fuckeliana* to *Botryotinia* and *S. candolleana* to *Ciborinia* on taxonomic grounds. Clearly if

Whetzel's taxonomic decision is not accepted (many plant pathologists and a few taxonomists do not) *Sclerotinia* may be treated in the broad sense of Fuckel to include a large and diverse group of plant pathogenic species.

Though taxonomic considerations afford some choice among the broad circumscription of Fuckel, the more restricted concept of Whetzel (1945) and Kohn (1979 a, b), there is no room for choice in considering the nomenclaturally correct name for *Sclerotinia sclerotiorum*. The genus *Sclerotinia* lectotypified in 1928 by Honey (1928) with *S. candolleana*. In 1945, Whetzel transferred *S. candolleana* to his new genus *Ciborinia*, and ignoring Honey's lectotypification, retypified his redelimited genus *Sclerotinia* with *S. sclerotiorum*. If one accepts Whetzel's restricted circumscription of genera (a taxonomic decision), *Sclerotinia* lectotypified by *S. candolleana*, becomes the oldest available name for *Ciborinia*. This was the position taken by Korf and Dumont (1972) in erecting *Whetzelinia*, typified by *S. sclerotiorum*. Because many workers, especially plant pathologists, accept both Whetzel's circumscription and his typification of *Sclerotinia* with *S. sclerotiorum*, a proposal to conserve *S. sclerotiorum* as the lectotype of *Sclerotinia* has been presented. It has been accepted by the special committee for Fungi and Lichens of the International Association of Plant Taxonomists (IAPT) and by the IAPT General Committee. However, routine action by the International Botanical Congress in 1981 is still pending. It is now correct to refer to *Sclerotinia sclerotiorum* and to ignore the generic name *Whetzelinia* henceforth.

7.3 Species Characters in *Sclerotinia*

Taxonomic decisions are based upon observation and evaluation of characters falling into four principal categories: macroscopic, cultural, biological and microscopic. The publication in 1932 of Nannfeldt's Studien fiber die Morphologie und Systematik der nicht lichenisierten inoperculaten Discomyceten (1932) revolutionized the description and classification of discomycetes by introducing micro anatomical studies of sterile tissues as a source of additional taxonomic characters. Using pre-Nannfeldt characters, as employed by many workers who described species of *Sclerotinia*, a description of a species was limited to the following range of characters:

1. Macroscopic characters, such as colour, size and shape of the apothecium, stipe and sclerotium. Cultural characters, often the size and distribution of sclerotia on agar plates.
2. Biological characters, such as host, season and part of substrate invaded.
3. Microscopic characters, usually limited to the size, shape and colour of the ascospores, asci and paraphyses.

While it must be noted that these characters are useful ones and indeed several have been heavily weighted in making the taxonomic decisions. The micro anatomical characters introduced by Nannfeldt in his classification offer further information on zones of the apothecium, stipe and sclerotium in addition to the hymenium, which has long been the center of attention. The sterile zones of the apothecium and sclerotium

show diverse and distinctive tissue types (Korf, 1973). These zones include the subhymenium, the medullary excipulum and the ectal excipulum subdivided into three component zones; the margin, the flank, the stipe and including any hairs, or as in the Sclerotiniaceae, tomentum hyphae.

The tissue types of the apothecial and sclerotial zones are characterized within the genus *Sclerotinia*. The subhymenium, a compact zone of interwoven prosenchyma is usually brown-walled and bound in gel. The medullary excipulum is composed of loosely interwoven textura intricata oriented more or less parallel to the surface of the apothecium. The most characteristic zone, the ectal excipulum is composed of textura prismatica which turns out at the apothecial margin perpendicular to the apothecial surface and further down the flank, develops into textura globulosa as cells become inflated, round off and become somewhat disarticulated. Globose cells and often tomentum hyphae occurring as processes growing from globose cells, comprise the ectal excipulum of the stipe and are often brown-walled. The sclerotial medulla in *Sclerotinia* does not include suscept tissues, but is composed of hyaline textura oblita with heavily gelatinized hyphal walls (composed of β-1, 3-g1ucans and proteins) as reported by Saito (1977). The sclerotial rind is composed of the apices of these medullary cells which turn out perpendicularly to the sclerotial surface and develop into textura prismatica, again with cells that become globose and somewhat disarticulated. Pigmentation of these rind cells may occur in the walls of a two to six deep layer of the outermost cells. All species retained in *Sclerotinia* show a positive reaction of the ascus pore channel wall in Melzer's Reagent (0.5 g iodine, 1.5 g potassium iodide, 20 g chloral hydrate and 20 ml distilled water). Dimorphism in spore size has been observed by Kohn (1979a) in one species as it has for some species of *Monilinia* (Woronin, 1888) and in *Sclerotinia allii* (Sawada, 1919), which is a species of *Ciborinia*.

7.4 Variability in Species Characters in *Sclerotinia*

The question of reliability of taxonomic characters has long been a disturbing one. Purdy (1955) studied variation in ascus, ascospore and sclerotium sizes in isolates tentatively identified as *S. sclerotiorum*, *S. trifoliorum and S. minor*. Comparing averages and ranges of ascus and ascospore measurements from two to three generations with those given in species diagnoses and elsewhere in the literature, Purdy found no line of demarcation between species and concluded that "it was impractical, if not impossible to distinguish the asci or ascospores of one species from those of another." Purdy obtained a variety of cultural variants within species ranging from those which produced no sclerotia to those which produced very large sclerotium-like masses. He found continuous intergradations in sclerotium size from the small sclerotia of *S. minor* to the large ones of *S. sclerotiorum*. Consequently he could not distinguish species on the basis of sclerotial size. On the strength of this evidence, without examining any type specimens and without examining apothecial tissue structure, Purdy synonymized not only *S. minor* and *S. trifoliorum* under *S. sclerotiorum*, but also

S. trifoliorum var. *fabae*, *S. intermedia* and *S. sativa* maintaining that it is "impossible to identify these species in practice because of the variability of the characters that have been used". Purdy's data may demonstrate the variability of the characters studied, but many more characters are available for consideration in species delineation. By reducing the number of species recognized it first appeared that the task of identifying plant pathogenic species of *Sclerotinia* would be simplified. As more was learned about the biology of this group, however, it became apparent that this broad definition of *S. sclerotiorum* seemed to submerge several taxa under one species.

Although certain cultural characteristics have been studied extensively by plant pathologists and taxonomists, in only a few cases apothecial structures have been examined for variation between apothecia produced in nature and those produced *in vitro*. Working with a collection of approximately 65 isolates of species of *Sclerotinia*, *Myriosclerotinia*, *Ciborinia* and *Botryotinia* from Europe, Australia, New Zealand, Asia and North America, Kohn (1979b) has observed cultural characteristics and apothecial production found for 26 per cent of these isolates. Apothecia produced *in vitro* were obtained only for isolates finally identified as *Sclerotinia sclerotiorum* (10 of 18 isolates), *S. minor* (5 of 7 isolates) and *S. trifoliorum* (2 of 5 isolates). Most isolates were derived from diseased tissue, although some were made from single ascospores or ascospore masses. Cultures grown and maintained on potato dextrose agar (PDA) were transferred to PDA in 9 cm diameter Petri plates, incubated for three to four days at room temperature, then transferred with a 5 mm diameter cork borer from the growing margin of the colony to 500 ml Erlenmeyer flasks containing autoclaved carrot discs and 25 ml of distilled water. The flasks were incubated for four weeks without light at 15°C. Sclerotia were harvested, rinsed in sterile distilled water and transferred to sterile Petri dishes containing glass wool saturated with distilled water. The sclerotia then were "cold conditioned" for four weeks at 0°C. The dishes were removed to a growth chamber, set at 15°C, with fluorescent and incandescent light at approximately 21,520 lux (2,000 ft-c) and a 14 h photoperiod. Apothecial initials appeared 4–12 weeks after introduction to the growth chamber. The apothecial initiation in *Sclerotinia* has been studied by many workers as reviewed by Saito (1977). According to Kohn (1979a) fertile apothecia were produced at 4,304 lux (400 ft-c) or above. At levels significantly below 4,304 lux only stipes were produced and just below this threshold level, apothecia developed only a pallisade layer of tomentum hyphae in place of a fertile hymenium.

Sclerotia and apothecia produced in nature by *S. sclerotiorum*, *S. minor* and *S. trifoliorum* were compared with apothecia and sclerotia produced *in vitro*. One isolate of *S. trifoliorum* was obtained from European collection of fresh apothecia from which a mass ascospore shoot was made, as were several isolates of *S. tuberosa*. Apothecia and sclerotia were sectioned at 20 and 5 μm, respectively. Sectioned apothecia were examined in Melzer's Reagent, cotton blue in lactophenol and KOH/phloxine only. In studying the cultural behavior of *S. borealis*, Groves and Bowerman (1955) reported that "the apothecia in the Swedish specimen (topotype) were smaller and more delicate than those developed in cultures of Kohn (1979a), who observed in other species of Sclerotineaceae that apothecia produced in culture are usually more robust than those formed in nature." Until Christiansen's (1966)

studies of variability in apothecial structure of several species of *Ciboria*, *Ciborinia*, *Lambertella*, and *Rutstroemia*, this observation had not been followed up by examination of sectioned apothecia to determine the histological relationship to this macroscopic variation. Variability in gross size of sclerotia and apothecia as well as in colour and external mealiness of apothecia has long been observed in both cultures and field collections of pathogenic species of *Sclerotinia* on examining sectioned apothecia. Kohn (1979b) found that this variation in size was not due to differences in cell size or in tissue types but as Christiansen noted in her studies, "basically the issue types of the different layers of the apothecia were stable, varying only in the compactness and in the admixtures present." Variation in gross size of apothecia of *Sclerotinia* appears to be due to the proliferation and compactness of cells. Only in the subhymenium are cells consistently bound in gel; in other zones of the apothecium, gel is present or not, with variation between isolates as well as among them. Since gel may be a factor in the conservation of moisture within apothecial tissue, presence or absence of gel is probably influenced by varying amounts of moisture in the microenvironment of the developing apothecium and should be approached with caution as a taxonomic character in this group of species.

The bluing of the ascus pore channel in Melzer's Reagent has long been accorded importance as a consistent taxonomic character by discomycetes taxonomists. Enhancement or initiation of this reaction after pretreatment with KOH has been reported by Kohn and Korf (1975) and Nannfeldt (1976). Though all species retained in *Sclerotinia* have ascus pore channels which turn blue in Melzer's Reagent, without KOH pretreatment. Most collections of *S. minor* showed a weak reaction or none at all. All reactions were enhanced or (in *S. minor)* occurred only after KOH pretreatment. No mention of the reaction is found in Jagger's original species diagnosis (Jagger, 1920). Bluing of sterile zones of the apothecium in Melzer's Reagent was observed in all species with variation in whether or not bluing occurred among isolates. Variability was not observed between field-collected apothecia and apothecia derived from isolates obtained from the same collection but developed *in vitro*. The apothecial zone that most often turned blue in Melzer's Reagent was the subhymenium and this reaction is probably due to the presence of gel in the layer, although not all apothecia with tissues bound in gel displayed this reaction. Variation in macroscopic colour of apothecia, including mottling is reflected in corresponding variation in pigmentation of cell walls in all zones of the apothecium. The ectal excipulum of the apothecium and stipe is the most frequently pigmented zone but cell walls in this zone may be hyaline, light brown, or dark brown with no consistency between or among isolates. External mealiness on the receptacle and stipe may be due to the presence of abundant tomentum hyphae to which soil particles may adhere. Occurrence and abundance of tomentum hyphae are extremely variable on the ectal excipulum of both the apothecium and the stipe, but tomentum hyphae are most frequently present on the stipe. While presence of tomentum in these areas cannot be relied upon as a taxonomic character in species retained in *Sclerotinia*, it does appear to be a more stable character of the sclerotial rind.

In comparing measurements of ascospores with those given in species diagnoses, variations of as much as 3 μm in range and average measurements made from a single apothecium were observed in different mounting media. Dilute solutions of chloral

hydrate and potassium hydroxide often are used to rehydrate and inflate dried specimens with collapsed cells and both also inflate ascospores. Since, most authors do not indicate the mounting medium in which measurements have been taken, workers should be aware of the effect of mounting media on ascospore and ascospore size, both of which are already subject to variability within a certain range in nature.

Cultures grown in PDA or autoclaved carrot discs at 15–20°C consistently produce either small sclerotia scattered throughout the colony or large sclerotia arranged radically at the periphery of the growing margin of the colony, consistent with whether original isolates are small sclerotial forms (*S. minor*) or large sclerotial forms (*S. trifoliorum* and *S. sclerotiorum*), respectively (Kohn, 1979a). Studies of sclerotial ontogeny by Willetts and Wong (1971) confirm this observation by reflecting a difference in the mode and location of sclerotial initiation among these species. Degeneration in the ability to produce sclerotia in isolates of these three species as well as in size and quantity of sclerotia produced has been observed with eventual loss of the ability to produce sclerotia occurring in some cultures. Although the failure of an isolate to produce sclerotia is often permanent in some cases. Formation of sclerotia may be induced by transferring the isolate to autoclaved carrot discs. Subcultures from the sclerotia produced on these carrot discs appear normal.

In recent years, several techniques previously not applied to comparative studies of the economically important species of *Sclerotinia* have been explored in an effort to supplement morphological data and shed light on the delimitation of biological species in this group. These techniques include comparative studies of cytology, electrophoretic assays, sclerotial ontogeny and mycelial interactions. Bjorling (1942, 1951) reported the nuclear number for ascospores of *S. sclerotiorum* and *S. trifoliorum* as two and four respectively, with the haploid chromosome number for both species of six. Frandsen (1946) reported that the haploid chromosome number of both species was eight. Later, Wong and Willetts (1979) have reported for *S. minor* and *S. trifoliorum* four nuclei per ascospore while *S. sclerotiorum* has two nuclei per ascospore. They also reported a haploid chromosome number for hyphal tips and germinating ascospores of four for *S. minor* and eight for *S. sclerotiorum* and *S. trifoliorum*. Kohn (1979a) confirmed these findings on nuclear numbers in studies of freshly produced apothecia. However, Berthet (1964) reported the nuclear number in ascospores of *S. tuberosa* to be two to six, whereas Kohn (1979a) observed two to four nuclei per ascospore.

On the basis of electrophoretic patterns for soluble proteins, aryl esterase, acid phosphatase, tetrazolium oxidase, glucose-6-phosphate dehydrogenate (NADP linked) and reduced nicotinamide adenine dinucleotide phosphate dehydrogenase of 47 isolates of *Sclerotinia* species collected from a variety of crops in Australia, New Zealand, North America and Europe, Wong and Willetts (1975) recognized four subgroups among their isolates:

Group 1: Small sclerotial isolates from a wide variety of host plants.
Group 2: Large sclerotial isolates from forage legumes.
Group 3: Large sclerotial isolates from a wide variety of host plants including forage and legumes.
Group 4: One isolate from *Anemone*.

It was interpreted by Wong and Willetts (1975) that these groups represent as *S. minor*, *S. trifoliorum*, *S. sclerotiorum* and *S. tuberosa* respectively and concluded that these were four distinct species with *S. tuberosa* "characteristically different" from the other three species (Plate 7.4.1). On the basis of morpholgical characters

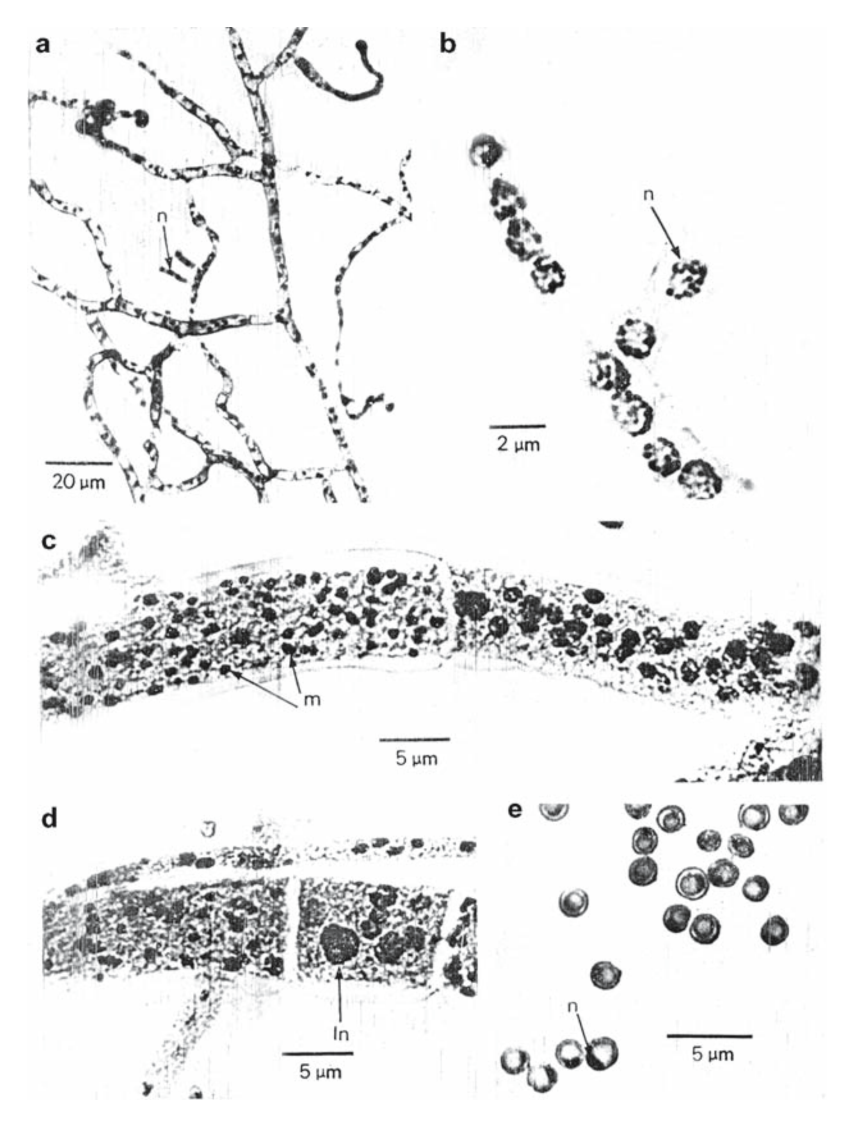


Plate 7.4.1 General cytological characteristics of *Sclerotinia* species. (a) Vegetative mycelium of isolate S3 (*S. minor*) showing the multinucleate condition of hyphal cells (n, nucleus); (b) Nucleus at hyphal tips of isolate S8 (*S. sclerotiorum*); (c): Large main vegetative hypha of isolate S8 showing large number of nuclei; the cell on the left shows synchronous mitosis of nuclei (m, mitotic fungus); (d): Different sizes of nuclei in a large hypha of isolate S8; Note the large nuclei (in); (e): Micro-conidia of isolate S7 (*S. trifoliorum*); each micro-conidium contain one nucleus (Adapted from the publication of Wong and Willetts, 1975. With permission)

Kohn (1979a) also recognized these species, retaining the first three in *Sclerotinia* and transferring *S. tuberosa* to a new genus, *Dumontinia*.

7.4.1 Generic Diagnosis

Sclerotinia Fuckel, Jahrb. Nassauischen Vereins *Naturk*. 23–24: 330. 1870 (Lectotype: *S. sclerotiorum* (Lib.) de Bary, typus conserv. proposition)

= *Ciboria* subg. *Sclerotinia* (Fuckel) Boud., *Bull. Soc. Myc. France* 1: 115. 1885
= *Whetzelinia* Korf & Dumont, *Mycologia* 64: 250. 1972 (nom Rejiclendum prop.)

Apothecia 2–10 mm wide, stipitate, produced from a sclerotium, receptacle cupulate to convex. Sclerotium produced free from host tissues, not incorporating host tissues within the sclerotial medulla. Asci eight-spored, J +, thin-walled, thickened at the apex. Ascospores uniseriate, smooth-walled, shape predominately ellipsoid, biguttulate. Subhymenium well developed, light brown-walled textura intricata, usually bound in gel. Medullary excipulum of hyaline, loosely woven textura intricata. Ectal excipulum of hyaline to light brown-walled textura prismatica, oriented perpendicularly to the apothecial surface with cells inflated to globose, tomentum hyphae often present. Microconidia produced superficially on cultures and on surface of hymenium, hyaline, globose, produced from single phialides borne laterally on hyphae, or grouped in sporodochia. Differing from other genera in the Sclerotiniaceae by the presence of a tuberoid sclerotium which does not incorporate host tissues and is borne superficially on aerial mycelium, absence of disseminative conidial state, production of hyaline ascospores, superficial production of micro conidia.

7.4.2 Morphology of Stroma

The stroma is food storage and survival organ composed of a hyaline hyphal medulla surrounded by a rind of cells with melanized walls. Whetzel (1945) recognized two types of stroma.

7.4.2.1 Substratal Stroma

It is an indeterminate stroma with a medulla of host tissue permeated by hyphae and with a thin black ring covering at least a portion of the stromatal surface.

7.4.2.2 Sclerotial Stroma

It is a determinate stroma either developing within host tissues and incorporating remnants of these tissues within the medulla, or developing free from the host tissues.

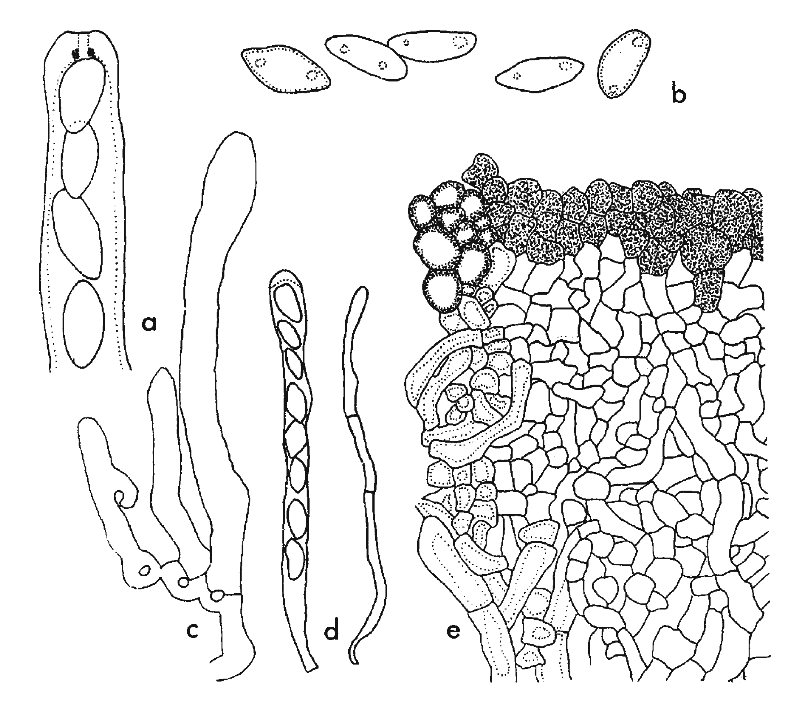


Plate 7.4.2.1 *Sclerotinia sclerotiorum* (a) Ascus with J + pore channel wall × 1,500; (b) Ascospores × 1,500; (c) Young asci arising from crosiers × 1,500; (d) Ascus and paraphyses × 500; (e) Cross section of sclerotial rind and medulla (Adapted from the publication of Kohn, 1979a. With permission)

The species of *Sclerotinia* possess a sclerotial stroma developing free from host tissues, not incorporating host tissues within the medulla and often collected in the absence of identifiable host organ remnants.

The sclerotial medulla of *Sclerotinia* is composed of hyaline textura oblita with heavily gelatinized walls, 2–3 μm thick. The sclerotial rind is composed of the apices of these medullary cells, which turn out perpendicularly to the sclerotial surface and become textura prismatica, with cells inflating to become globose and often somewhat disarticulated. Brown pigmentation of these rind cells occurs in the walls of a two to six deep layer of the outermost cells (Plates 7.4.2.1, 7.4.2.2). Sclerotia of *Sclerotinia* species develop abundantly in culture although the ability to produce sclerotia may be lost in old isolates. Kohn (1979a) observed sclerotial development superficially above the surface of the agar on aerial mycelium.

7.4.3 Microconidia

As in other genera of the Sclerotiniaceae, globose, hyaline microconidia are produced from flask-shaped phialidic conidiophores borne laterally on hyphae either

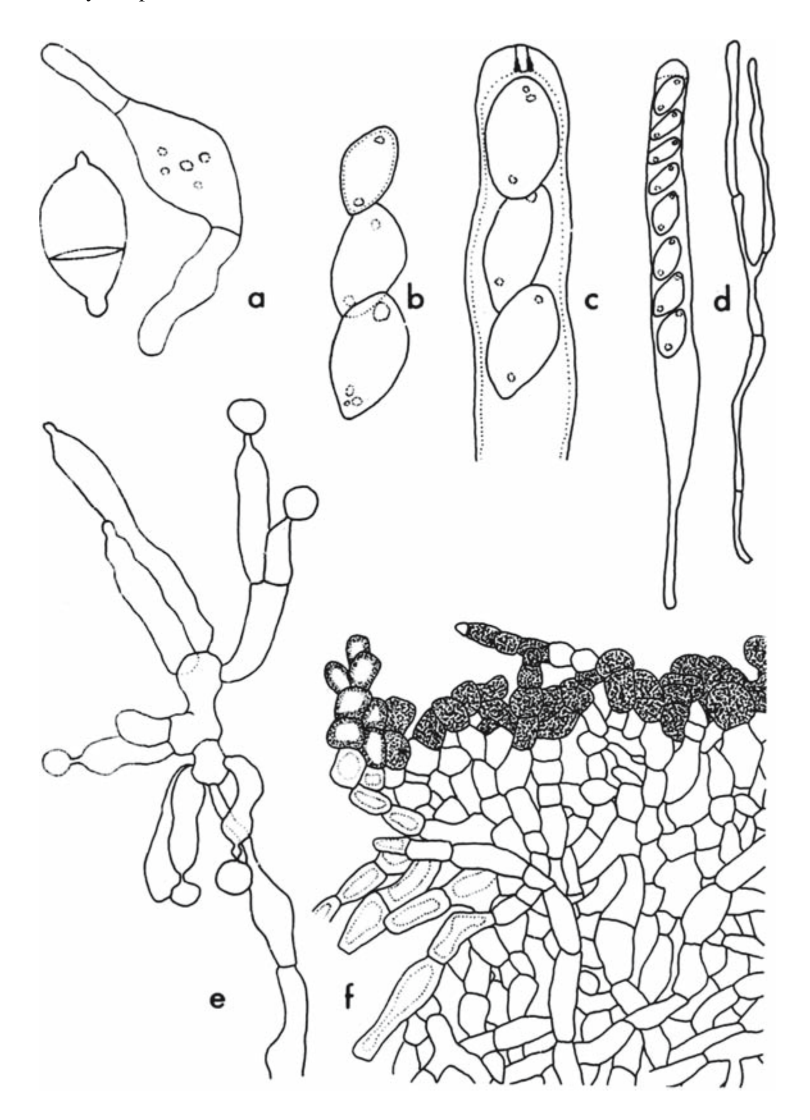


Plate 7.4.2.2 *Sclerotinia trifoliorum* (a) Germinating ascospores × 1,500; (b) ascospores × 1,500; (c) Ascus with j + pore channel wall × 1,500; (d) Ascus and paraphyses × 500; (e) Myrioconium microconidial state, young conidiophore in developing sporodochium produced on aerial hyphae in culture × 1,500; (f) Cross section of sclerotial rind and medulla (Adapted from the publication of Kohn, 1979a. With permission)

singly or grouped in sporodochia (Plate 7.4.2.2). In *Sclerotinia* microconidia are produced superficially on aerial mycelium in culture, on the hymenial surface of apothecia (from germinating ascospores) and on the surface of sclerotia. Since the species of *Sclerotinia* are homothallic (Keay, 1939), the role of microconidia as functional spermatia is dubious. Evidence has been given for microconidia serving

as germinable spores after over wintering in *Botrytis fabae* (Harrison and Hargreaves, 1977) but as yet no such role has been proven for species in *Sclerotinia*.

7.4.4 Ascocarp

The apothecia are stipulate, copulate and are produced from a sclerotium (Plates 7.4.4.1, 7.4.4.2). Stipe primordia originate within the medulla of the sclerotium and eventually rupture the rind (Saito, 1977). Apothecia are 2–10 mm in diameter, cinnamon to amber and usually concolorous, though mottling and darkening at the margins are common. Some mealiness due to tomentum hyphae may be present on the surface of the receptacle and stipe. The eight-spored asci are produced from vertically oriented repeating croziers (Plates 7.4.2.1, 7.4.2.2). The asci are cylindrical tapering down to the attachment to the crozier. The ascus walls are thin, 1–2 μm thick (Plates 7.4.2.1, 7.4.2.2, 7.4.4.2). Species in *Sclerotinia* show a bluing reaction of the ascus pore channel wall in Melzer's Reagent, termed J + . In one species this reaction is very weak but is enhanced with pretreatment in 2 per cent KOH following the procedure of Kohn and Korf (1975). The ascospores are hyaline and ellipsoid to somewhat flattened on one surface (Plate 7.4.4.3). In one species dimorphism in spore size has been observed, generally with a 4:4 segregation of small and large ascospores within the ascus (Plate 7.4.4.3). This phenomenon has been observed and illustrated by Woronin (1888) and Buchwald (1956) in *Monilinia* and by Sawada (1919) in *Ciborinia allii*. Ascospores are uniseriate in the ascus (Plate 7.4.4.3). Germination of ascospores may be bipolar or unipolar, with germination often occurring from the middle of the ascospores (Plate 7.4.4.3). In culture, ascospores are biguttulate at maturity. Nuclear number within ascospores varies from two to four according to species as reported by Wong and Willetts (1979) and verified by Kohn (1979a).

The paraphyses are filiform, sparsely septate and occasionally branched (Plate 7.4.4.3). The subhymenium is a compact layer, usually bound in gel of pale brown-walled textura intricata. This layer often blues in Melzer's Reagent. The medullary excipulum is composed of loosely interwoven textura intricata, usually hyaline and often incorporating rhomboidal crystals. The ectal excipulum of the apothecium and stipe is composed of textura prismatica oriented perpendicularly to the apothecial surface, with the hyaline to pale brown-walled cells becoming inflated to globose and disarticulating somewhat. In one species, the margin of the apothecium is composed completely of globose cells (Plate 7.4.4.4). In the other species, the margin is composed of textura porrecta parallel to the asci; further down the ectal excipulum towards the flanks, the textura porrecta develops shorter cells oriented perpendicularly to the apothecial surface. Gel may be present or absent in this layer (Kohn, 1979a) and bluing in Melzer's Reagent occurs occasionally in the presence of the gel. Tomentum hyphae, one to two cells in length are often present as processes

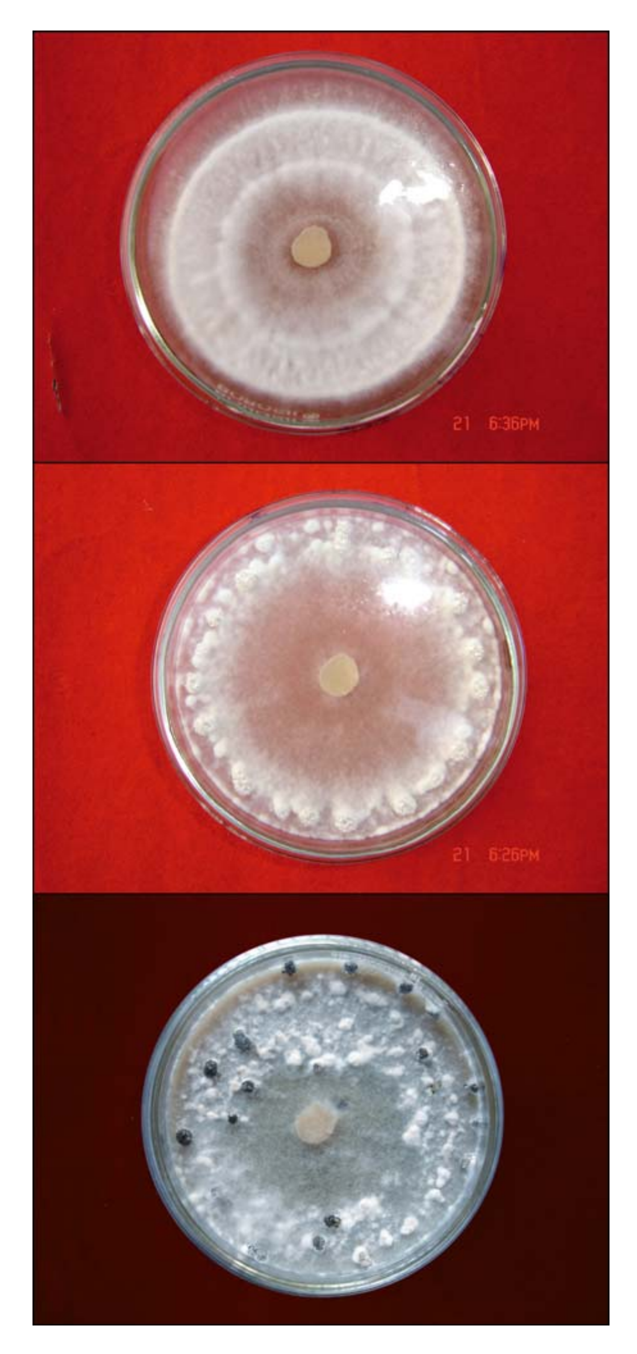


Plate 7.4.4.1 Growth pattern of *Sclerotinia sclerotiorum* (a) Mycelial growth; (b) Initaition of sclerotia formation; (c) Sclerotia formation at the outer periphery (*See Color Plates*)



Plate 7.4.4.2 Apothecia formation in *Sclerotinia sclerotiorum* (*See Color Plates*)

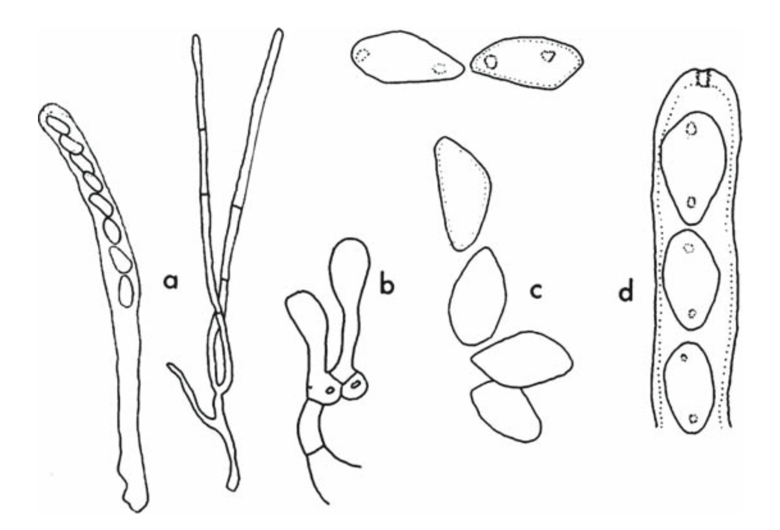


Plate 7.4.4.3 *Sclerotinia minor* (a) Ascus and paraphyses × 500; (b) Young asci arising from crosiers × 1,500; (c) Ascospores × 1,500; (d) Ascus with J + pore channel wall × 1,500 (Adapted from the publication of Kohn, 1979a. With permission)

from the globose cells of the ectal excipulum of the apothecium, stipe and sclerotium. These are usually hyaline to pale brown-walled on the apothecium and darker brown and often grouped into fascicles on the stipe. The presence or absence of tomentum appears to be rather variable (Kohn, 1979a) but is of some use as a taxonomic character of the sclerotial rind (Plate 7.4.4.5).

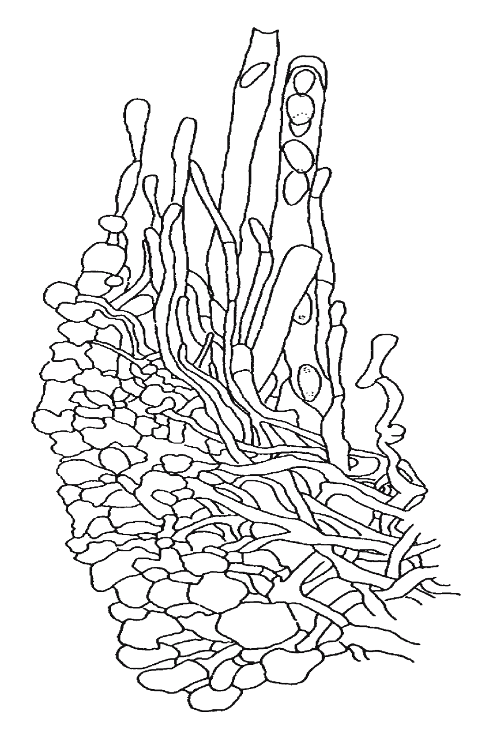


Plate 7.4.4.4 Cross section of the margin of the apothecia × 500; *S. minor* the ectal excipulum at the margin is composed of globose cells (Adapted from the publication of Kohn, 1979a. With permission)

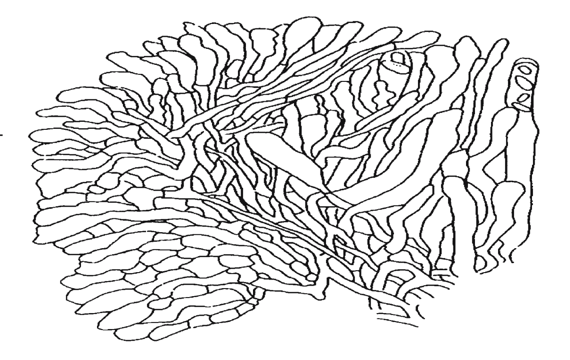


Plate 7.4.4.5 Cross section of the margin of the apothecia × 500; *Sclerotinia sclerotiorum* the ectal excipulum at the margin is composed of prosenchyma "turning out" perpendicularly to the apothecial surface (Adapted from the publication of Kohn, 1979a. With permission)

7.5 Key to the Sclerotium Forming Genera of the Sclerotineaceae (Kohn, 1979a)

1. Apothecia copulate or verpoid, stipitate, on a distinct sclerotium with a well differentiated rind and medulla; conidia produced not *Sclerotinia* (cfr. *Phaeosclerotinia*, *Monilinia*, *Pycnopeziza*, *Scleromitrula*, *Botryotinia*, *Gloeotinia*, *Septotinia*).

1. Apothecia copulate or verpoid, stipitate, on a distinct sclerotium with a well differentiated rind and medulla; conidia absent except for phialidic microconidia *(Myrioconium)*..2
   2. Ascospores brown..*Martininia*
   2. Ascospores hyaline..3
3. Sclerotial medulla enveloping suscept tissues or, in culture, sclerotia at least partially immersed in the agar..4
3. Sclerotial medulla free of suscept tissues or, in culture, sclerotia formed above the agar surface..7
4. Ectal excipulum of apothecium composed of prosenchymatous cells ....*Elliottinia*
4. Ectal excipulum of apothecium composed of globose cells..............................5
5. Sclerotia and microconidia produced within the stems of graminaceous, juncaceous, and cyper aceoushosts..*Myriosclerotinia*.
5. Sclerotia and microconidia produced within the stems of other monocot and dicot families..6
6. Apothecia verpoid.. *Verpatinia*.
6. Apothecia copulate.. *Ciborinia*.
7. Stroma consisting of mantling stroma of indefinate dimensions and smaller sclerotia (sclerotules) formed on aerial mycelium above the mantle; apothecia occurring on the mantling stroma only ....*Stromatinia*.
7. Not as above..8
   8. Apothecia verpoid..*Mitrula sclerotiorum*. (a presumed parasite on sclerotia of *Sclerotinia* spp.)
   8. Apothecia cupulate 9
9. Outer layer of apothecial ectal excipulum composed of prosenchymatous cells usually embedded in a gel ....*Dumontinia*.
9. Outer layer of apothecial ectal excipulum composed of globose cells ....*Sclerotinia*.

7.6 Key Leading to the Plant Pathogenic Species of *Sclerotinia*, Based on Sclerotia Producing (Cultures Grown on PDA at 15–20°C and on Field-Collected Sclerotia (Kohn, 1979a))

1. Sclerotia and mycelium with one or both of the following characters:

   (a) clamp connections; (b) dolipore septa (use phase microscopy or mounts in aniline blue/glycerine (Tu and Kimbrough, 1973)..........................Basidiomycetes

1. Sclerotia and mycelium with neither of the above characters................. 2
2. Conidia present ...Not *Sclerotinia*

(Cfr. *Verticillium*, *Phaeosclerotinia*, *Monilinia*, *Pycnopeziza*, *Scleromitrula*, *Botryotinia*, *Gloeotinia*, *Septotinia*, *Cristulariella*, etc.).

2. Conidia absent, except for phialidic "spermatia" *(Myrioconium)*.....................3
3. A mantling sclerotial stroma of indefinite dimensions present and smaller sclerotia ("sclerotlutis" formed on aerial mycelium above the mantle………………
…*Stromatinia*.
3. Not as above……………… 4
4. Sclerotial medulla containing host cells (n.b. vessel elements with spiral cell wall thickenings), or in culture, sclerotia at least partially immersed in the agar. ………………………………………*Ciborinia*,*Myriosclerotinia*,"*Sclerotinia" kerneri*.
4. Sclerotial medulla free of suscept tissues, or in culture, sclerotia formed above the agar surface……………………………………………………………..5
5. Sclerotial rind a single layer of dark-walled, clavate cells. …………*Sclerotinia tuberosa*.
5. Sclerotial rind composed of a two to six deep layer of dark-walled, globose cells……………..6
6. Sclerotia formed abundantly, scattered throughout colony, sometimes adhering to form an aggregate crust in culture; individual sclerotia 0.5–2 mm long……………*S. minor*.
6. Sclerotia produced at growing margins of colony only, forming concentric rings, radial lines and other patterns; individual sclerotia 2–20 mm long………………………..7
7. Sclerotial rind composed of textura prismatica with cells becoming globose, continuing beyond the rind as erect, tomentum hyphae…………………………… *S. trifoliorum*.
7. Sclerotial rind composed of textura prismatica with cells becoming globose, no tomentum hyphae present……………………………………………………… *S. sclerotiorum*.

7.7 Key Leading to the Sclerotium-Forming Plant Pathogenic Species of *Sclerotinia* Based on Apothecia with Sclerotia Produced *In Vitro* or in Nature (Kohn, 1979a)

1. Apothecia cupulate, stipitate, on a distinct sclerotium with a well-differentiated rind and medulla, conidia present……..……………………………………..… Not *Sclerotinia*
1. Apothecia cupulate, stipitate on a distinct sclerotium with a well-differentiated rind and medulla, conidia absent, except for phialidic spermatia (*Myrioconium*)……………………………………………………………2

2. Sclerotial medulla containing host cells, or, in culture, sclerotia at least partially immersed in the agar............................*Ciborinia*, *Myriosclerotinia*, *"Sclerotinia" kerneri*.
2. Sclerotial medulla containing host cells, or in culture, sclerotia formed above the agar surface...3
3. Stroma consisting of a mantling stroma of indefinite dimensions and smaller sclerotia formed on aerial mycelium above the mantle; apothecia occurring on the mantling sclerotial stroma only ....*Stromatinia*.
3. Not as above 4
4. Outer layer of ectal excipulum of apothecia composed of prosenchymatous cells usually embedded in gel..*Sclerotinia tuberosa*.
4. Ectal excipulum composed of globose cells, gel present or absent.......... *Sclerotinia* (5)
5. Ascospores dimorphic in size, showing segregation in ascus, tetranucleate, length/width ratio of ascospores <2.0.. ....*S. trifoliorum*.
5. Ascospores uniform in size, no segregation in ascus 6
6. Ectal excipulum at margin of apothecium composed of globose cells, ascospores tetranucleate...*S. minor*.
6. Ectal excipulum at margin of apothecium composed of prosenchyma "turning out" perpendicularly to the apothecial surface; ascospores binucleate, length/width ratio of ascospores >2.0...................................*S. sclerotiorum*.

7.8 Key to the Plant Pathogenic Species Included in *Sclerotinia* (Kohn, 1979a)

1. Ascospores dimorphic in size, showing segregation in ascus, Tetranucleate, length/width ratio of ascospores <2.0; ectal excipulum at margin of apothecium composed of prosenchyma "turning out" perpendicularly to the apothecial surface; sclerotia produced at growing margins of colony only, forming concentric rings, radial lines and other patterns, individual sclerotia 2–20 mm long ....*Sclerotinia trifoliorum*.
1. Ascospores uniform in size, no segregation in ascus... 2
2. Ectal excipulum at margin of apothecium composed of globose cells; ascospores tetranucleate length/width ratio of ascospores < or >2.0; sclerotia formed abundantly throughout the colony, sometimes adhering to form an aggregate crust in culture, individual sclerotia 0.5–2 mm long ....*S. minor*.
2. Ectal excipulum at margin of apothecium composed of prosenchyma "turning out" perpendicularly to the apothecial surface, length/width ratio of ascospore 2.0, ascospores binucleate; sclerotia produced at the growing margins of colony only, forming concentric rings, radial lines and other patterns, individual sclerotia 2–20 mm long....*S. sclerotiorum*.

7.9 Accepted Species

7.9.1 Sclerotinia sclerotiorum

(Lib.) de Bary, Vergl. *Morph. Biol. der Pilze, Mycet. Bact*., p. 22. c. i.e., 1884.

= *Peziza sclerotiorum* Lib., Plant. Crypt. Ard. 326. 1837. (!!)

= *Helotium sclerotiorum* (Lib.) Fuckel, *Fung. Rhen. Exsicc. Suppl. IV*. 1861. 1866.

= *Sclerotinia libertiana* Fuckel, Jahrb. *Nassauischen Vereins Nuaturk*. 23–24: 331. 1870 (name change).

= *Phialea sclerotiorum* (Lib.) Gillet, Chamb. France discomyc. p.98, c. i.e., 3 (1). 1881.

= *Hymenoscypha sclerotiorum* (Lib.) Phillips, Man. Brit. Discomyc., p.115. 1887.

= *Whetzelinia sclerotiorum* (Lib.) Korf & Dumont, *Mycologia* 64:250. 1972; [*Whetzelinia sclerotiorum* Wellman, *Dictionary of Tropical American Crops* and *Their Diseases*, p. 10, 1977 (*Lapsus calami*)]; [*Sclerotinia whetzelinia* Wellman, *Dictionary of Tropical Plant Crops and their Diseases*, p. 395. 1977 (*Lapsus calami*).]

= *[Peziza sclerotii* Fuckel, Bot. Zeit. (Berlin) 35:249, c. i.e.-x (a-d). 1861 (*nomen nudum*).]

= *Peziza kauffmanniana* Techomirow, *Bull. Soc. Imp. Naturalistes Muscou* 4: 295, c. i.e.- IV-VII. 1868.

= *Rutstroemia homoeocarpa* Karst., Bildrag. Kannedom Finlands, *Natur Folk* 19: 107. 1871. (!!).

= *Sclerotinia postuma* Berk.& Wilson, *Gard. Chron*. 20: 333, c. i.e. 1883.

= *Sclerotinia ficariae* Rehn in Rabenh., Krypt. Fl. *Deutschl.* 11. 1 (3): 815. 1893. (!!)

= *Sclerotinia opuntiarum* Speg., *Anales Soc.C i. Argent*.50: 37. July, 1900 (!!),

= *Sclerotinia sclerotiorum* (Lib.) de Bary var. opuntiarum (Speg.) Allipi, Revista Fac. Agron. Univ. Nac. La Plata 36:149. 1960.

= *Sclerotinia moelleriana* P. Henn.., *Hedwigia* 41: 27. 1902 (!!)

= *Sclerotinia wisconsinensis* Rehm., *Ann. Mycol*. 6: 317. 1908. (!!)

= *Sclerotinia matthiolae* Lendn., *Bull. Soc. Bot. Geneve* 9: 21, c. i.e.-1–3, 1917; *Bull. Soc. Bot. Geneva* 9: 221. 1918.

= *Sclerotinia henningsiana* Kirschst., Verb. *Bot. Provo Brandenburg* 40: XXVII. 1918. (!)

= *Sclerotinia riograndensis* Rick, Broteria, Ser. Bot. 25: 99. 1931 (!)

= *Sclerotinia galeopsidis* Velen., Monogr. Discomyc. Bohem. 1: 227. 1934 (!!)

= *Sclerotinia caudata* Velen., Novitates Mycol. Novissimae P. 129. 1947. (!!)

= *Sclerotinia sclerotiorum* (Lib.) de Bary forma *Orobanches* Narasimhan & Thirumalachar, Phytopath. Z. 22: 426, c. i.e. 1954.

= *Sclerotinia xanthorrhoeae* Beaton & Weste, Trans. Br. Mycol. Soc. 68: 73, c. i.e. 1977. (!!)

Sclerotia borne superficially, usually on dense, white mycelium, globose to cylindrical but quite variable in shape, (2–) 5–15 (–30) × 2–8 (–15) mm, with black outer rind and white inner context; in culture developing at the growing margins of the

colony, often forming in concentric rings, radiating lines and other patterns. Sclerotial medulla of tightly interwoven, hyaline textura oblita, cells 5–10 μm wide with heavily gelatinized walls 2–3 μm thick. Sclerotized rind a two to six cell, deep layer of textura prismatica originating from medullary cells turning out perpendicularly to the surface, becoming brown walled, inflated to globose, 5–15 μm in diameter. Apothecia arising one to several from a sclerotium, ochraceous (- cinnamon) to amber, often darker at the base of the stipe; receptacle 2–8 (−10) mm broad, applanate to slightly concave when young, at maturity applanate to convex, often with a central depression, frequently with an undulate margin, tapering to form a stipe 3–20 (−30) mm long, 1–2 mm wide. Ectal excipulum 35–140 μm broad, thin-walled hyaline to pale brown textura prismatica with cells inflated to globose, 5–30 μm wide, sometimes bound in gel, oriented perpendicularly to the apothecial surface, at the margin a brown-walled textura porrecta with inflated apices; outermost excipular cells sometimes giving rise to one to two celled tomentum hyphae; ectal excipulum of stipe composed of light brown-walled textura porrecta with one-celled, inflated tomentum hyphae arising from the outermost cells and turning out perpendicularly from the stipe axis. Medullary excipulum of loosely woven, thin-walled, hyaline textura intricata, hyphae 5–15 μm wide, occasionally bluing in Melzer's Reagent. Sometimes in two layers, an outer layer of textura porrecta parallel to the apothecial surface, and an inner layer of textura intricata; medullary excipulum of stipe composed of textura porrecta or of textura oblita with walls 1–2 μm thick; rhomboidal crystals often abundant in receptacle and/or stipe. Subhymenium of compact or loosely woven light brown-walled textura intricata, sometimes bluing in Melzer's Reagent and probably bound in gel, hyphae 2–3 μm wide. Asci arising from croziers, eight-spored, cylindrical, (110−) 130–150 (−160) × 6–10 μm, with a thickened apex, pore channnel wall J +. Ascospores uniseriate, hyaline, ellipsoid, biguttulate, binucleate, (9−) 10–14 × 4–5 (−6) μm, length/width ratio usually greater than 2.0. Paraphyses hyaline, filiform, 2 μm wide, septate, simple or sparsely branched. Microconidial state (*Myrioconium*) with microconidia globose, hyaline, 2–4 μm in diameter produced from phialides in sporodochia or on phialides borne laterally on hyphae, superficial on hymenium surface or culture (Kohn, 1979a). The detailed information on habitat, type locality, type specimen, typical host of basionym, typical hosts of synonyms, exsiccati specimens examined, other specimens examined with and without apothecia of this species has been given by Kohn (1979a).

7.9.2 Sclerotinia minor

Jagger, J. Agric. Res. 20: 333, c. i.e. 15. XI. 1920.

= *Sclerotinia intermedia* Ramsey, Phytopathology 14: 324, c. i.e. 1924 (!!)
= *Sclerotinia sativa* Drayton & Groves, Mycologia 35: 526. 1943. (!!)

Sclerotia borne superficially, irregularly shaped, 0.5–2 (−5) mm in diameter, with black outer rind and white inner context, in culture forming abundantly throughout the colony, sometimes adhering to form an aggregate crust of indefinite

dimensions. Scleroial medulla of tightly interwoven, hyaline textura oblita, cells 5–10 µm thick. Sclerotial rind a two to six deep layers of textura prismatica originating from medullary cells turning out perpendicularly to the surface, becoming brown-walled, inflated to globose, 5–15 µm in diameter. Apothecia arising singly from a sclerotium, cinnamon to amber, sometimes darker at base of stipe; receptacle 2–9 mm broad, cupulate to applanate when young, at maturity applanate with a central depression, margin undulate or somewhat incurved, tapering to form a stipe 1–4 (−12) mm long and 1–2 mm wide that is often broader at the apex than at the base. Ectal excipulum 40–100 µm broad of thin-walled, hyaline to pale brown textura prismatica with cells inflated to globose, 5–35 µm in diameter, sometimes bound in gel, more often only cells at margin bound in gel, oriented perpendicularly to the apothecial surface; outermost excipular cells often giving rise to one to two celled tomentum hyphae; ectal excipulum of stipe composed of light brown, thin walled textura prismatica turning out perpendicularly to the stipe axis, cells 5–35 µm in diameter, giving rise to one to two celled tomentum hyphae which are sometimes grouped into fascicles. Medullary excipulum of loosely woven, thin-walled, hyaline textura intricata, hyphae 5–20 µm wide; medullary excipulam of stipe composed of textura porrecta parallel to the stipe axis; rhomboidal crystals, often in clusters, usually abundant, especially in medulla of stipe. Subhymenium a compact zone, 15–40 µm broad of pale brown-walled textura intricata, usually bound in gel, often turning blue in Melzer's Reagent, hyphae 2–3 µm wide.

Asci arising from croziers, cylindrical, eight-spored, (110–) 125–180 × 7–11 µm with a thickened apex, without pretreatment in 2 per cent KOH pore channel wall weakly J +. Ascospores uniseriate, hyaline, ellipsoid, biguttulate, tetra-nucleate, 8–17 (−20) × (4) 5–7 (−9) µm, length/width ratio greater or less than 2.0. Paraphyses hyaline, filiform, 2 µm broad, widening slightly to 3 µm at apices, septate, simple to sparsely branched. Microconidial state (*Myrioconium*) with microconidia globose, hyaline, (2–) 3–4 µm in diameter, produced from phialides in sporodochia or on phialides borne laterally on hyphae, superficial on hymenium surface or culture (Kohn, 1979a). Detailed observations on habitat, type locality, type specimen, typical hosts of basionym, typical hosts of synonyms, exsiccati specimens examined, other specimens examined with and without apothecia have been given by Kohn (1979a).

7.9.3 Sclerotinia trifoliorum

Erikss., Landtbruks - Akad. Handi *Tidskr*. 19: 28. 1880. (!!)

= *Peziza ciborioides* Hoffm. non Fr. In Rabenh., F. *Europ. exsicc*. 619. 1864 (later homonym of *P. ciborioides* Fr. ex Fr., *Syst. Mycol*. 2 (1): 117. 1822). (!!)
= *Sclerotinia bryophila* Kirschst., *Ann. Mycol*. 36: 381. 1938. (!!).
= (*Sclerotinia trifolii* Biffen, J. Roy. *Agr. Soc. England* 97: 482. 1936 (*Lapsus calami*).]

Misapplications: Peziza ciborioides Fr. ex Fr., by Hoffman Jones Anal. Fung. 111, P. 65. 1861; by E. Rehm, J. Landw. 20:151–178, c. i.e. −1, 2.1872 – by H. Rehm,

Ascomyceten 107. 1872. Sclerotia borne superficially, globose to cylindrical, 2–12 (−20) × 2–8 mm, with black outer rind and white inner context; in culture developing at the growing margins of the colony, often forming in concentric rings, radiating lines and other patterns. Sclerotial medulla of tightly interwoven, hyaline textura oblita, cells 5–10 µm wide with heavily gelatinized walls 2–3 µm thick. Sclerotial rind composed of textura prismatica originating from medullary cells turning out perpendicularly to the surface, becoming brown-walled, inflated to globose, 5–15 µm in diameter, continuing past rind as erect, multicelled, brown-walled tomentum hyphae. Apothecia arising one to several from a sclerotium. Greyish septa to amber, concolorous though margin and base of stipe may be darker; receptacle 3–7 (−10) mm, applanate to slightly concave when young, at maturity cupulate with a central depression, often with an undulate margin, tapering to form a stipe 3–15 (−28) mm long and 1–2 mm wide that is often broader at the apex than at the base. Ectal excipulum 50–125 µm broad, thin-walled, hyaline to pale brown walled textura prismatica with cells becoming inflated to globose, 5–20 µm in diameter, oriented perpendicularly to the apothecial surface, at the margin a brown-walled textura porrecta with inflated apices; outermost excipular cells often giving rise to one to two celled tomentum hyphae; ectal excipulum of stipe composed of light brown-walled textura prismatica parallel to the stipe axis with one celled inflated tomentum hyphae arising from the outermost excipular cells and turning out perpendicularly to the stipe axis. Medullary excipulum of loosely woven, hyaline, thin-walled textura intricata, hyphae 5–10 µm wide; outer zone often bound in gel, often bluing weakly in Melzer's Reagent; medullary excipulum of stipe composed of hyaline textura porrecta parallel to the stipe axis. Subhymenium a compact zone 15–20 µm broad of light brown-walled textura intricata, hyphae 2–3 µm wide, usually turning blue in Melzer's Reagent and bound in gel. Asci arising from croziers, eight-spored, cylindrical, 140–200 × 10–12 µm with a thickened apex, pore channel wall distinctly J^{+}. Ascospores uniseriate, hyaline, ellipsoid to allantoid, biguttulate, tetranucleate, 10–20 × (4−) 6–9 (−11) µm, dimorphic in size and segregating within the ascus (usually 4:4), larger spores mostly 13–18 × 7–9 µm, smaller spores mostly 10–13 × 6–7 µm, length/width ratio usually less than 2.0, two celled in age. Paraphyses hyaline, filiform, with apices slightly inflated, 3 µm wide, septate, simple or sparsely branched. Microconidial state (*Myrioconium*) with microconidia globose, hyaline, 2–3 µm in diameter, produced from phialides in sporodochia or on phialides borne laterally on hyphae, superficial on hymenial surface or culture (Kohn, 1979a). Other details on habitat, type locality, type specimen, typical host of basionym, typical hosts for synonyms, exsicatti specimens examined, other specimens examined with and without apothecia of the species have been given by Kohn (1979a).

7.10 Taxa Imperfecti Known

Kohn (1979a) has mentioned 259 taxa under this head. According to Kohn (1979a), the diagnoses are insufficient to determine the species, or in some case the appropriate generic position. The detailed taxa have been published in *Mycotaxon* 9: 365–444.

7.11 Economically Important or Often Cited Species Excluded from *Sclerotinia*

Sclerotinia allii is transferred to *Ciborinia* as *Ciborinia allii* (Saw.) Kohn. comb. novo (basionym: *Sclerotinia allii* Sawada, Govt. Formosa Agric. Exp. Stn., Spec. Bull. 19: 206. 1919). Yamamoto et al. (1956) by error thought that Sawada's species produces a *Botrytis* state, which is identified as *B. byssoidea* Walker, calling the species *Botryotinia allii* (Saw.) Yamam. Unpublished cultural studies by R. P. Korf and G. L. Hennebert show that Sawada's species has no conidial state. The combination in *Botryotinia* is now unfortunately well-established in the literature.

Sclerotinia camelliae Hara non Hansen & Thomas belongs either in an unnamed new genus or may be a species of *Moellerodiscus*.

Sclerotinia camelliae Hansen & Thomas non Hara is a *Ciborinia*. This epithet was published after 1935 without a Latin diagnosis and is, therefore, not validly published. However, Kohn (1979a) provided a Latin diagnosis for it in 1979.

Sclerotinia fructicola (Wint,) Rehm is *Monilinia fructicola* (Wint.) Honey.

Sclerotinia fructigena Aderh. & Ruhl. is *Monilinia fructigena* Honey.

Sclerotinia fuckeliana (de Bary.) Whetz. is *Botyotinia fuckeliana* (de Bary.) Whetz.

Sclerotinia gladioli Drayton is *Stromatinia gladioli* (Drayt.) Whetz.

Sclerotinia homoeocarpa Bennett has no existing type specimen and is not a *Sclerotinia*. The epithet has been applied to species with apothecia identified as belonging to *Lanzia* sp. and to *Moellerodiscus* sp. The symptoms attributed to "dollar spot" probably are caused by more than one species.

Sclerotinia kerneri Wettstein belongs in a new genus (Kohn, 1979a).

Sclerotinia laxa Aderh. & Ruhl. is *Monilinia laxa* (Aderh. & Ruhl.) Honey.

Sclerotinia narcissicola Gregory is *Botryotinia narcissicola* (Qreg.) Buchw.

Sclerotinia panacis Rankin is transferred to *Stromatinia* as *Stromatinia panacis* (Rank.) Kohn, comb. nov. (basionym: *Sclerotinia panacis* Rankin, Phytopathology 2: 30. 1912)

Sclerotinia perplexa Lawrence is *Ovulinia perplexa* (Lawr.) Seav.

Sclerotinia ricini Godfrey is *Botryotinia ricini* (Godfr.) Whetz.

Sclerotinia serica is transferred to *Stromatinia* as *Stromatinia serica* (Keay) Kohn. comb. Nov. (basionym: *Sclerotinia serica* Keay. J. Bot. 75: 132. 1937).

Sclerotinia tuberosa (Hedw. ex Merat) Fekl. belongs to a new genus (Kohn, 1979a).

7.12 Description of Species

7.12.1 Sclerotinia fuckeliana

Hosts: Occurs both as a parasite and a saprophyte on a very wide range of host plants.
Disease: Causes 'grey mould' or 'botrytis disease', a blight or rot of immature, fleshy or senescent tissues. Lesions develop as tan or brown water soaked areas,

which may become greyish on drying out. The profuse grey brown sporulation of the fungus on old diseased tissue is characteristic. Rotting of perishable plant produce at harvest or in store causes large losses; can be particularly severe on soft fruit such as strawberries, grapes and vegetables such as cabbage, lettuce etc. Damping-off and basal leaf and stem rot result in severe damage to lettuce and flax. Blights of buds, blossom, leaves and stems may also occur on a wide range of hosts and the fungus has been implicated in dieback and canker formation on woody plants.

Geographical distribution: Worldwide, but mostly prevalent as a disease in humid temperate or sub-tropical areas.

Transmission: Conidia are air-borne, but may be carried on the surface of rain splash droplets. Diseased plant parts, on which sporulation is profuse in wet weather are important sources of inoculum in disease epidemics. The fungus over winters as sclerotia or as mycelium in old plant debris and may be seed borne as spores or mycelium on e.g., flax. IMI Descriptions of Fungi and Bacteria, 1998 (44), Sheet 481 (Ellis and Waller, 1998).

7.12.2 Sclerotinia sclerotiorum

Hosts: The fungus is plurivorous; hosts include *Brassica oleracea* var. *capitata*, *B. chinensis*, *Helianthus annuus*, *Phaseolus vulgaris*, *Arachis hypogaea*, *Carthamus tinctorius*, Citrus, *Coriandrum sativum*, *Cucumis melo*, *Curcurbita pepo*, *Glycine max*, *Lycopersicon esculentum*, *Nicotiana tabacum*, *Lactuca sativa*, *Hibiscus sabdariffa*, *Cucumis sativus*.

Disease: The fungus causes diseases with several common names such as cottony soft rot, white mould and watery soft rot. It is generally more important as a pathogen of vegetables in the field during transit and in store. Woody plants, grasses and cereals are rarely attacked. Crops attacked include cabbage (*Brassica oleracea* var. *capitata* and *B. chinensis*); sunflower (*Helianthus annuusi*); common bean (*Phaseolus vulgaris*); groundnut (*Arachis hypogaea*); safflower (*Carthamus tinctorius*); citrus (*Citrus spp.*); coriander (*Coriandrum sativum*); melon (*Cucumis melo*); squash (*Curcurbita pepo*); soybean (*Glycine max*); tomato (*Lycopersicon esculentum*); tobacco (*Nicotiana tabacum*); lettuce (*Lactuca sativa*); roselle (*Hibiscus sabdariffa*); cucumber (*Cucumis sativus*). Most plant parts, above and at soil level of herbaceous crops can be attacked at any age. The first symptoms are frequently the collapse of the plant due to stem infection near the soil. A soft rot develops, followed by the conspicuous, external white mycelium and the sclerotia which are often formed in the pith. Infections which arise at some height above soil level frequently begin from withering or fallen petals infected by ascospores.

Geographical distribution: Very widespread but the disease is in one of relatively cool moist conditions areas. In the Mediterranean area little or no disease occurs in the summer. Transmission: Air-borne ascospores are the most important means of spread. The sclerotia (from which the apothecia arise) are the primary survival structures, in soil and host debris. Survival time is very variable but can be high

after three years in soil (Hoes and Huang, 1975). Mycelium from sclerotia can also cause infection. Seed may be an infective source, either from contaminating sclerotia or internal mycelium. IMI Descriptions of Fungi and Bacteria, 1998, Sheet 513 (Mordue and Holliday, 1998).

7.12.3 Sclerotinia fructicola

Hosts: On almond, apple, apricot, cherry, cherry plum hybrids, nectarine, peach, pear, plum, *Prunus* spp. and grapevine.

Disease: Brown rot of stone fruits especially of plum, cherry, peach nectarine and apricot, quince and occasionally of apple and pear; black apples. Blossom wilt important on plum, cherry, peach and nectarine; twig and spur blight, canker, on these hosts and on apple and quince.

Geographical distribution: Japan, Australia, New Zealand, Canada, USA, Central America, Argentina, Bolivia, Peru, Venezuela. There are records from S. Africa and from Egypt. CMI Map 50, ed. 4, 1976.

Transmission: Conidia formed on over wintered mummified fruits and other infected organs and disseminated by wind and rain are the major source of infection. Apothecia form relatively frequently and ascospores are capable of initiating infection, nitidulid and certain other beetles act as vectors and conidia are carried by bees at flowering. IMI Descriptions of Fungi and Bacteria, 1998 (62): Sheet 616 (Mordue, 1998a).

7.12.4 Sclerotinia fructigena

Hosts: On almond, apple, apricot, cherry, fig, loquat, medlar, nectarine, peach, pear, persimmon, plum, quince, grapevine, *Amelanchier canadensis*, barberry, blackberry, cherry laurel, *Cornus mas*, Corylus, Cotoneaster, *Crataegus oxyacantha*, *C. subvillosa*, Fragaria, *Prunus divaricata*, *P. spinosa*, *Pyrus purpureum*, *P. sieboldii*, *Sorbus aucuparia*, *S. dacica*, Vaccinium.

Disease: Brown fruit rot particularly of apple, pear, plum, cherry, peach, nectarine, apricot, quince, black apples, less frequently, twig blight and canker.

Geographical distribution: Widespread in Europe and Asia, also present in Egypt, Morocco, Brazil, Chile and Uruguay. CMI Map 22, ed. 4, 1976.

Transmission: Mummified fruits over winter either on trees or on the ground beneath and at the start of the growing season give rise to sporodochia and infrequently, apothecia. In addition conidia form on other infected tissues such as cankers and blighted twigs. Spores are disseminated by air currents and water splash. There is limited spread by mycelial growth. IMI Descriptions of Fungi and Bacteria, 1998 (62): Sheet 617 (Mordue, 1998b).

7.12.5 Sclerotinia homoeocarpa

Hosts: On turf grasses: *Agrostis alba*, *A. canina*, *A. palustris*, *A. stolonifera*, *A. stolonifera* subsp. *compacta*, *A. tenuis*, Avena, *Cynodon dactylon*, *Digitaria didacryla*, *Festuca ovina*, *F. rubra*, *F. rubra* subsp. *comutata*, *F. rubra* var. *fallax*, *Holcus lanatus*, Lolium, *Paspalum notatum*, *Pennisetum clandestinum*, *Poa annua*, *P. pratensis*, *P. trivialis*, *Puccinellia maritima*, *Cyperus rotundus*. By inoculation on radish, lettuce, beet, tomato, subterranean clover, pea, bean, wheat seedlings, barley and onion.

Disease: Dollar spot of turf. The spots are about 5 cm diameter and approximately circular, though they sometimes coalesce to form irregular patches. They are brown at first, later becoming bleached to straw coloured. The fungus is present on leaves, stems and basal parts of the plants, which eventually die.

Geographical distribution: Britain and Northern Ireland, Netherlands, Australia, New Zealand and USA.

Transmission: No detailed studies made. The fungus is known to be capable of over wintering in individual dollar spots. Dissemination by infected leaf fragments has been reported. IMI Descriptions of Fungi and Bacteria, 1998, Sheet 618 (Mordue, 1998c).

7.12.6 Sclerotinia laxa

Hosts: On almond, apple, apricot, cherry, *Corylus avellana* Fragaria, loquat, medlar, nectarine, peach, pear, plum, *Prunus* spp., *Pyrus* spp., quince, rhododendron and grapevine.

Disease: Brown fruit rot particularly of plum, cherry, peach, nectarine, apricot and quince, less commonly of apple and pear, black apples. Blossom wilt, wither tip, spur blight, twig blight, canker, leaf infection. Considered to be a pathogen of blossom and twigs more than fruit.

Geographical distribution: Morocco, S. Africa, Afghanistan, China, Israel, Japan, Lebanon, Turkey, USSR (Central Asia), Australia, New Zealand, widespread in Europe, Canada, USA, Guatemala, Argentina, Brazil, Chile, Uruguay. CMI Map 44, ed. 4, 1976.

Transmission: Mummified fruits over winter either on trees or on the ground beneath and at the start of the growing season give rise to sporodochia and less frequently, apothecia. Conidia also form on other infected organs such as twig cankers and blighted spurs. Conidia and ascospores are capable of initiating infection and are disseminated by air currents and water splash. There is a limited amount of spread by vegetative mycelium. IMI Descriptions of Fungi and Bacteria, 1998, Sheet 619 (Mordue, 1998d).

7.12.7 Sclerotinia borealis

Hosts: *Lolium perenne* and various cereals, forage grasses and lawn grasses (including *Triticum cultivars*, *Phleum pratense*, *Agrostis tenuis*, *Alopecurus pratensis*,

Anthoxanthum odoratum, *Bromus inermis*, *Dactylis glomerata*, *Festuca* spp., *Poa pratensis*, *Secale cereale*). According to Saito and Tkachenko (2003), the fungus has a host range extending over 82 species in 50 genera of 18 plant families including three species of monocots.

Disease: Snow scald, snow mould.

Geographical distribution: Asia, Japan, USSR, Europe, Eurasia, Finland, Norway, Sweden, USSR, North America, Canada (Alberta, British Columbia, Manitoba, Saskatchewan, Yukon), United States (AK, MN, WA). IMI Distribution Maps of Plant Diseases, No. 446.

Transmission: Penetration of the fungus has been shown to occur (*in vitro*) through stomata and intercellularly. In the field disease entry can be facilitated by injury which is increased by slight freezing of the soil, a thick snow cover and slow melting of the snow in the spring. Sclerotia develop within the culms, digesting and to some extent incorporating the host tissue. Sclerotia may also be present on the leaves. Germination of sclerotia occurs to produce apothecia, with the subsequent production of ascospores which may then become the infective agents. The development of apothecia and the dissemination of ascospores are favoured by long, rainy autumns. IMI Descriptions of Fungi and Bacteria, 1998 (109): Sheet 1082 (Williams and Spooner, 1998a).

7.12.8 Sclerotinia narcissicola

Host: *Narcissus* spp.

Disease: Smoulder, grey mould. Infection may reduce bulb yield and flower size. Symptoms may include, rot of the bulbs and leaves at ground level, brown lesions on the leaves and flower buds, distortion and failure of emergence.

Geographical distribution: Asia, Iraq, USSR, Australasia, Australia (Tasmania, Victoria), New Zealand, Europe, Channel Islands (Guernsey, Jersey), Denmark, Eire, England, Germany, Northern Ireland, The Netherlands, Norway, Scotland, Sweden, USSR, Wales, West Germany, North America, Canada (British Columbia, NS, Ontario, PEI), USA (North Carolina, New York, Oregon, Virginia, Washington State). IMI Distribution Maps of Plant Diseases, No. 315.

Transmission: The disease may come from planting of infected bulbs or from infected soil, sclerotia in the soil may be viable for up to nine months. *In vitro* conidial suspensions does not cause infection except of wounded or damaged tissue, mycelial inoculation consistently caused lesions on detached leaves and bulb scales. IMI Descriptions of Fungi and Bacteria, 1998, Sheet 1083 (Williams and Spooner, 1998b).

7.12.9 Sclerotinia trifoliorum

Hosts: *Trifolium* spp., also *Medicago sativa* and other herbaceous leguminous forage crops including *Anthyllis vulneraria*, *Lathyrus* spp., other *Medicago* spp.,

Melilotus spp. and *Vicia* spp. including *V. faba* (on this host causing 'Bean rot'; the causal agent of which has often been referred to as *S. trifoliorum* var. *fabae* Keay) and *V. sativa*. Although approximately 100 hosts have been recorded for this pathogen, there is often a lack of conclusive evidence that *S. trifoliorum* and not another *Sclerotinia* species is to blame.

Disease: Rot, called variously Stem rot, Crown rot, Brown patch of lawns, Clover sickness, Clover canker. Symptoms include leaf rot, petiole rot and stem rot. Initial leaf spotting may be followed by these more severe rot symptoms. The foliage usually turns grey-green as though scalded, then may wither and the rot may spread. In Lucerne, the leaves may be totally destroyed by the pathogen, but it takes a long time to reach the root system through the comparatively thick stem.

Geographical distribution: Africa, Egypt, Asia, China, India, Israel, Japan, Korea, Australasia & Oceania, Australia (NSW, Vietnam, Tas., W.A.), New Zealand, Europe, Austria, Belgium, Bulgaria, Czechoslovakia, Denmark, Eire, Finland, France, Germany, Greece, Hungary, Italy, The Netherlands, Norway, Poland, Romania, Sweden, Switzerland, UK, USSR, North America, Canada (Alberta, British Columbia, Manitoba, Que, PEI), USA (widespread), Mexico, Central & South America, Chile.

Transmission: The development of apothecia occurs in the autumn. Ascospores infect the leaves and rotting of the clover plants sets in the following early spring. The fungus is able to complete its entire life-cycle as a saprophyte. Spread from plant to plant takes place chiefly along affected petioles, but the pathogen may grow about 2 cm over the soil from a nutritional base. The fungus can persist in the crown of the plant throughout the summer until harvest. Sclerotia may germinate to produce apothecia and ascospores which may infect emergent shoots, sclerotial germination is favoured by light, well-aerated soils and a temperature between 10°C and 20°C. Mycelium and ascospores remain viable (in a dry state) for seven months, sclerotia buried in the soil survive for more than seven years. *In vitro* conidia will infect clover plants. (IMI Distribution Maps of Plant Diseases No. 274.) IMI Descriptions of Fungi and Bacteria, 1998, Sheet 1084 (Williams and Spooner, 1998c).

7.13 New Species of *Sclerotinia*

7.13.1 Sclerotinia nivalis sp. *nov.*

A new species of *Sclerotinia*, previously reported as *S. intermedia* from Japan is described as *S. nivalis* on the morphological basis of the sclerotial anamorph and teleomorph produced in culture. The characters assigning this species to the genus *Sclerotinia* are the tuberoid sclerotia superficially produced on suscepts, the small sclerotia produced on aerial mycelium in culture, the interhyphal spaces in medullary tissue of sclerotia and the globose cells constructing the ectal excipulum of apothecia. It can be distinguished from *S. sclerotiorum*, *S. minor* and *S. trifoliorum* by the intermediate sized sclerotia in culture, binucleate ascospores, the molecular mass of major proteins of sclerotia and the patterns of esterase isoenzymes in sclerotial extracts. Although *S. nivalis* causes snow mould of various dicots, it is a

mesophile having an optimum temperature for mycelial growth of around 20°C. It attacks edible burdock (*Arctium lappa*), *Chrysanthemum morifolium* [*Dendranthema morifolium*], *Ambrosia elatior* [*A. artemisiifolia*], carrot (*Daucus carota*), *Angelica acutiloba*, *Ajuga reptans* and *Plantago lanceolata* (Saito, 1997).

7.13.2 Sclerotinia ginseng sp. *nov.*

This recently described species is isolated from *Panax pseudoginseng* in Liaoning, China. It differs from other known *Sclerotinia* spp. in morphology and pattern of soluble protein, pectinesterase and polygalacturonase (Wang et al., 1995).

7.13.3 Sclerotinia glacialis sp. *nov.*

It is described from leaf bases of *Ranunculus glaciales* at Switzerland (Graf and Schnumacher, 1995).

7.13.4 Sclerotinia trillii sp. *nov.*

Sclerotinia trillii sp. *nov.*, which attacks *Trillium tschonoskii* and *T. smallii* in Hokkaido and northern Honshu, Japan is described. The characters identifying this species with the genus *Sclerotinia* are large tuberoid sclerotia, produced both on infected plants and in culture, which consisted of only mycelium (true sclerotia) and flesh apothecia produced on them. This species is distinguished from *S. sclerotiorum*, *S. minor*, *S. trifoliorum* and *S. nivalis* by relatively large sclerotia, cultural colony appearance and red-brown to yellow-brown, relatively large apothecium, in addition to its parasitic nature on *Trillium. Sclerotinia trillii* is a psychrophilic having an optimum temperature for mycelial growth at 15–20°C (Narumi et al., 2001).

7.14 Cultural and Biochemical Characteristics for Distinguishing *Sclerotinia* Species

The revised taxonomy of *Sclerotinia* retains only three species within the genus, i.e., *S. sclerotiorum* (Lib.) de Bary, *S. minor* Jagger and *S. trifoliorum* Erikss. Specific nomenclature is still based largely upon morphological and physiological criteria such as mycelial growth rates or the dimensions, morphology (macro and micro anatomical) and growth characteristics of sclerotia, asci and ascospores *in vitro*. In some cases the host association of a particular isolate in the field has been used as important criteria for species identification, particularly when differentiating *S. trifoliorum* from *S. sclerotiorum*. Using these traditional approaches some

workers, e.g., Jagger (1920), Keay (1939), Whetzel (1945), Bjorling (1951), Williams and Western (1965a), Buchwald and Neergaard (1973), Kohn (1979a) and Willetts and Wong (1980) supported the separation of *S. sclerotiorum*, *S. minor* and *S. trifoliorum* whilst others e.g., Purdy (1955), Walker (1969), Morrall et al. (1972) and Price and Calhoun (1975b) believed that it should be included in the single species *S. sclerotiorum*, their judgment being based upon the conclusion that the evidence available from such traditional taxonomic methods was unreliable and inadequate. During recent years several workers (Willetts and Wong, 1971; Wong and Willetts, 1973, 1974, 1979; Scott, 1981c; Russo et al., 1982; Peterson et al., 1982; Cruickshank, 1983) have carried out extensive investigations into the taxonomy of *S. sclerotiorum* and closely related species, using novel criteria, such as ontogenetic and cytological diversity and electrophoretic and serological data. Results of such studies have supported the view that *S. sclerotiorum*, *S. trifoliorum* and *S. minor* are three distinct species.

A range of cultural and biochemical criteria have been compared to assess their value in distinguishing species of *Sclerotinia* by Tariq et al. (1985). Twenty-seven isolates of *Sclerotinia* have been included with representatives from all three species groups recognized in recent taxonomic treatment. When mycelial characteristics are compared, growth rate at 25°C distinguishes *S. trifoliorum* from *S. sclerotiorum* and *S. minor*, while assessments of relative hyphal densities and mycelial interactions yield results with only limited taxonomic value (Fig. 7.14.1; Tables 7.14.1, 7.14.2). Comparison of sclerotial characteristics differentiate

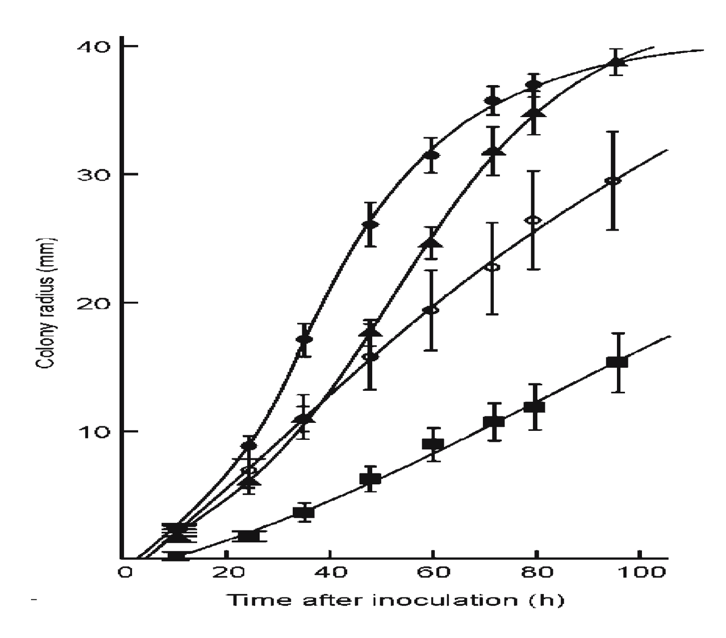


Fig. 7.14.1 Growth curves for *Sclerotinia* isolates (●) *S. sclerotiorum* (Ss1–Ss18); (○) Ss 19 & Ss 20; (▲) *S. minor* (Sm 25–Sm 27); (■) *S. trifoliorum* (St 21–St 24) (Adapted from the publication of Tariq et al., 1985. With permission)

Table 7.14.1 Summary of gross mycelial characteristics (Adapted from the publication of Tariq et al., 1985. With permission)

	On solid media (PDA)		In liquid media (CAT)		
Isolate	Colony radium after 48 h (mm)	Relative density of aerial mycelium	Pellet diam after seven days (mm)	Dry wt. of mycelium after seven days (mg)	General appearance of pellet after seven days
Ss1 to Ss18	26.1 ± 1.7 (55)	Sparse-very dense	38.3 ± 1.7 (29)	527.3 ± 70.2 (17)	White to cream no sclerotia
Ss19 and Ss20	15.8 ± 2.6 (8)	Dense–very dense	13.8 ± 3.2 (4)	120.5 ± 65.5 (2)	Creamy-white to grayish white single sclerotium observed on one pellet
St21 to St 24	6.3 ± 1.0 (15)	Sparse	10.0 ± 1.3 (6)	87.8 ± 5.5 (4)	White to cream immature sclerotia
Sm25 to Sm 27	17.6 ± 1.0 (11)	Moderately dense	21.0 ± 1.9 (5)	216.3 ± 32.9 (3)	White to cream mature and immature sclerotia

Mean ± S.E.M.; Numbers in parentheses represent the number of values used in calculating the mean

Table 7.14.2 Summary of sclerotial characteristics (Adapted from the publication of Tariq et al., 1985. With permission) (After three weeks on 15 ml PDA, at 25°C in the dark)

Isolate	Diam. of sclerotium (mm)	No. of sclerotia per 9 cm Petri dish	Wt. of dried sclerotium (mg)	Sclerotial patterns on solid media	Conc. of protein in sclerotia (mg/g fresh wt.)
Ss1 to Ss18	3.04 ± 0.04 (1.0–9.5)	27.0 ± 1.2	5.4 ± 0.8	Concentric circles throughout colony and/or at end of plate	30.8 ± 1.3
Ss19 and Ss20	4.2 ± 0.1 (2.0–7.8)	9.8 ± 1.3	7.9 ± 0.6	Concentric circles	38.9 ± 7.3
St21 to St 24	3.67 ± 0.12 (10–16.0)	26.6 ± 2.4	6.7 ± 2.3	Concentric circles within colony or radiating lines from inoculum plug	19.7 ± 1.1
Sm25 to Sm 27	1.48 ± 0.04 (1.0–3.0)	411.2 ± 1.3	0.4 ± 1.0	Scattered throughout colony. No distinct pattern	30.3 ± 3.1

Mean ± S.E.M. = Numbers in parentheses represent range of values

S. minor from the other two species on the basis of sclerotial size and pattern within the colony, but does not distinguish *S. sclerotiorum* from *S. trifoliorum*. Also spore characteristics are not considered useful because the time factor and unpredictability involved in ascocarp production precludes their use for rapid identification. Comparison of major soluble proteins from sclerotia by polyacrylamide gel electrophoresis, or discriminant analysis of pyrolysis mass spectra of sclerotial preparations, adds further support to species groupings construct using traditional cultural criteria. It will be difficult to use biochemical criteria alone to distinguish between the three species. Much closer similarities exist between *S. sclerotiorum* and *S. trifoliorum* than between both fungus and *S. minor* and it is suggested that they may be better regarded as subspecies (Tariq et al., 1985).

7.15 Cytology

Cytological studies carried out by Wong and Willetts (1979) indicated that isolates of *S. minor* have four nuclei in each ascospore and a haploid chromosome count of four while *S. trifoliorum* has four nuclei in each ascospore and a haploid chromosome count of eight. But *S. sclerotiorum* has two nuclei per ascospore and a haploid chromosome count of eight. Thus, consistent cytological differences support that *S. minor*, *S. trifoliorum* and *S. sclerotiorum* are distinct species.

7.16 Genetics and Molecular Aspects

A 1,380bp intervening sequence within the mitochondrial small sub-unit rRNA (nst ssu rRNA) gene of *S. sclerotiorum* has been sequenced and identified as group 1-intron. The intron shows a close similarity in secondary structure to the subgroup-IC2 introns from *Podospora* (ND 3i1, ND 5i2, and CO Ii5) and *Neurospora* (ND 5i1). Screening by means of Southern hybridization and PCR amplification detected the intron in the mt ssu rRNA gene of *S. minor*, *S. trifoliorum* and *S. cepivorum*, but not in other members of the Sclerotineaceae (Carbone et al., 1995). *S. sclerotiorum* can be transformed with polyethylene glycol-mediated protoplast transformation but the absence of reproductivity and stability of transformants combined with the multinucleate nature of hyphal cells have allowed relatively few moleculear studies of the pathogen (Rolland et al., 2003). Recently the first *Agrobacterium tumefaciens* mediated transformation of *S. sclerotiorum* was reported through co-cultivation with ascospores (Weld et al., 2005). Utilizing the benefits of *Agrobacterium* mediated transformation of bi-nucleate ascospores, future gene transfer, gene knock out and insertional mutagenesis studies on this pathogen should expedite. Nonetheless, the utility of reporter genes such as the green fluorescent protein (GFP) gene have already been exploited in *S. sclerotiorum* to gain more understanding on the biology of the fungus both *in vitro* and *in planta* (De Silva et al., 2005; Guimaraes and Stoz, 2004; Lorang et al., 2001).

The 38-Mb genome of *S. sclerotiorum* was sequenced through support from the US department of Agriculture by the Broad Institute of Harvard and the Massachusetts Institute of Technology. Based on shotgun sequencing, the current assembly contains 679 sequence contigs giving 8X sequencing coverage of the genome (*Sclerotinia sclerotiorum* Sequencing Project:http://www.broad.mit.edu/ annotation/ fungi/ sclerotinia/ sclerotiorum).Trace sequence have been deposited at the National Centre for Biotechnology Information (NCBI). The assembled sequence was released in the autumn of 2005. The publication of the full genome sequence opens a new chapter in research on this important pathogen (Bolton et al., 2006).

7.17 Electron Microscopy

Based on microanatomy, it is possible to differentiate *S. sclerotiorum* from two related species according to the number of rind layers of sclerotia and according to the size of the spores in the ascus. The rind of the sclerotium in *S. sclerotiorum* is composed of only two layers of rind globular cells. In the asci, eight ascospores of the same size are formed. No other differences are found between the structure of the reproductive organs of *S. sclerotiorum* and those of related species (Ziman, 1997).

The microanatomy of immature ‘white’, slightly pigmented and mature, one month old ‘black’ sclerotia of *S. trifoliorum*, *S. sclerotiorum* and *S. minor* has been studied through SEM by Arseniuk and Macewiez (1994b). A surface mycelial network is present over sclerotia at maturity. Also dried exudate on the superficial, sclerotial cells at maturity is observed. At this stage of morphogenesis an outer layer of the wall of medullary hyphae is synthesized. Two zones i.e., rind and medulla of hyphal tissue in sections of mature sclerotia is distinguished. The wall of rind cells is thick and one layered whereas the wall of medullary hyphae is thick and bi-layered. No lacunae (intercellular spaces) in sclerotial rind are found but the sclerotial medulla appears to be lacunate in all three species. At the SEM level, the structural organization of sclerotia of *S. trifoliorum* is identical to that one of sclerotia of *S. sclerotiorum* and *S. minor*. Thus, a unique, structural characteristic of taxonomic importance to distinguish *S. trifoliorum* from other *Sclerotinia* spp. has not been observed. However, through SEM study in all the three species, in the sterile tissue zones of apothecia, the only inter-specific difference detected is that one in the structure of the apothecial margin of small and large sclerotium. The ectal excipulum, at the apothecial margin in *S. minor* appears to be comprised of textura porrecta with prosenchymatous cells. The other inter-specific difference is dimorphism in spore size (ratio 4:4) in asci of *S. trifoliorum*. Otherwise the microanatomy of ascospores of *S. trifoliorum* and *S. sclerotiorum* is identical to that one of *S. minor*. The differences in the density of hyphae in the medullary excipulum in the apothecial discs of the three species have not been detected. The presence of a membranous film over the hymenium of the ascocarp of *S. trifoliorum* or any other spp. as well as the bundle-like structure of apothecial stipes as described earlier has not been observed by Arseniuk and Macewez (1994a).

7.18 Identifying New Characters for *Sclerotinia* Taxonomy

This is a watershed period in fungal systematics. Relatively few mycologists are doing taxonomy, i.e., developing systems of classification, making expert identifications, describing nomenclaturally significant taxa and preparing monographic and floristic studies. Kohn (1992) suggested some sources for new characters in fungal systematics which can be used for *Sclerotinia* taxonomy to resolve the disputed points.

New characters	Expected resolution level
Morphological	
Histochemistry	Species, genus, family
Ultrastructure	Any level
Anamorph connections	Genus, family
Anamorph morphology	Species, genus
Genetic	
Ability to mate and form viable F^1	Species
Vegetative incompatability	Intra-specific
Mycelial intersterility	Species
Biological	
Host or substrate	Inter-specific, species, genus
Biogeography	Any level
Molecular – Proteins – Immunology	Any level
– Sequencing	Any level
– Isozyme electrophoresis	Population, intra-specific, species
DNA – Restriction analysis – RFLPs	Intra-specific, species (any level)
– Restriction mapping	Any level
PCR – Length polymorphism	Any level
– Restriction analysis	Any level
Direct sequencing	Any level
RAPD	Intra-specific (genetic)

However, Ekins et al. (2005) suggested comparison of characters like host species, sclerotial diameter, ascosporic morphism and breeding type and RFLP probes for separating *S. minor* from *S. sclerotiorum* and *S. trifoliorum*.

7.19 Phylogeny of *Sclerotinia* and Related Genera

Phylogenies have been constructed based on nuclear ribosomal internal transcribed spacer (ITS) DNA sequences from an in-group consisting of 50 isolates representing 24 species of the discomycete family Sclerotiniaceae and an out-group consisting of five related taxa of the same family. The ingroup taxa are: three *Botrytis* spp., two *Botryotinia* spp., one *Ciborinia* sp., one *Dumontinia* sp., one *Grovesinia* sp.,

six *Myriosclerotinia* spp., nine *Sclerotinia* spp. and one *Sclerotium* sp. The outgroup taxa are: one *Ciboria* sp., one *Encoelia* sp. and three *Monilinia* spp. The type species is included for all taxa except for *Ciborinia* and *Encoelia*. Several of the included taxa are important plant pathogens. The resulting phylogenies are discussed with regard to morphology, life history and taxonomy. A suspected relationship between *Sclerotinia borealis* and *S. tetraspora*, and *Myriosclerotinia* is rejected, while a suspected relationship between *Ciborinia ciborium* and *Myriosclerotinia* is strongly supported. *Sclerotinia ulmariae*, previously synonymized with *Dumontinia tuberosa*, is reinstated as an independent species of *Dumontinia*. Two new combinations, *Dumontinia ulmariae* and *Myriosclerotinia ciborium* are proposed. The imperfectly known taxon *Sclerotium cepivorum* seems most closely related to *Dumontinia*. It is concluded that *Dumontinia* and *Myriosclerotinia*, as currently conceived, are monophyletic and that *Botryotinia* along with *Botrytis* anamorphs probably also constitute a monophyletic lineage. The genus *Sclerotinia* is probably polyphyletic and characterized by simple isomorphies rather than synapomorphies. Two putatively new taxa, *Sclerotinia* sp.1 and *Sclerotinia* sp. 2 are most closely related to *S. minor*, *S. sclerotiorum* and *S. trifoliorum* and to *S. borealis*, respectively (Holst-Jensen et al., 1998).

Chapter 8
Reproduction and Reproductive Structures

The life cycle of *Sclerotinia* spp. can be completed on a variety of artificial and synthetic media. As a result, numerous articles reporting various aspects of growth and morphogenesis of *Sclerotinia* spp. have been published. The formation and germination of dark-coloured, hardened compact masses of fungal tissue known as sclerotia have been studied in numerous fungi. From such studies, certain similarities and differences have been noted. Depending on the manner in which hyphae aggregate, the main types of sclerotium development are classified as terminal, strand or loose.

8.1 Sclerotia

Sclerotia play a major role in disease cycles as they produce inoculum and are the primary long-term survival structures (Willetts and Wong, 1980) remaining viable for up to eight years in soil (Adams and Ayers, 1979). A sclerotium is a hyphal aggregate with an outer black rind several cells thick containing melanin, a compound that is believed to play an important role in protection from adverse conditions and microbial degradation in many fungi (Bell and Wheeler, 1986; Henson et al., 1999) and in some cases function in virulence, e.g., in *Magnaporthe* and *Colletotrichum* species, mutants that do not accumulate melanin are unable to form well developed appressoria and incapable of penetrating the host, although mutants unable to form melanin in *Alternaria alternata* and *Venturia inaequalis* retain their virulence (Fitzgerald et al., 2004; Howard and Valent, 1996; Thomma, 2003). No association between melanin and virulence has been found for *S. sclerotiorum*. The inner portion of the sclerotium, the medulla is imbedded in a fibrillar matrix and is composed of carbohydrates, primarily β-glucans and proteins (LeTourneau, 1979). Sclerotia of *S. sclerotiorum* vary dramatically in size depending on the host. On sunflower, e.g., a *Sclerotium* covering the seed layer may be 1 cm thick and exceed 35 cm in diameter while on dry bean, the sclerotia may be globose and 2–10 mm in diameter.

Three stages of sclerotial development have been characterized (Townsend and Willetts, 1954); (i) initiation (aggregation of hyphae to form a white mass called

G.S. Saharan, Naresh Mehta, *Sclerotinia Diseases of Crop Plants: Biology, Ecology and Disease Management*,

sclerotial initials), (ii) development (hyphal growth and further aggregation to increase size), and (iii) maturation (surface delimitation, melanin deposition in peripheral rind cells and internal consolidation). An extensive list of environmental and nutritional factors that influence sclerotial development has been compiled (Chet and Henis, 1975; LeTourneau, 1979; Willetts and Bullock, 1992; Willetts and Wong, 1980). In general sclerotia are produced after mycelail growth encounters a nutrient limited environment (Christias and Lockwood, 1973). In culture medium, pH has been shown to have a significant influence on sclerotial development. Under neutral or alkaline pH, sclerotial formation is inhibited (Rollins and Dickman, 2001). Conversely, progression of oxalic acid accumulation by the fungus leads to lowering of environmental ambient pH (Maxwell and Lumsden, 1970) which provides conditions that favour sclerotial development (Rollins and Dickman, 2001). In support of this hypothesis, *S. sclerotiorum* mutant unable to produce oxalic acid are also unable to produce sclerotia *in vitro* and hence, non pathogenic *in planta* (Dickman and Mitra, 1992; Godoy et al., 1990). However, the ability to develop sclerotia is not restored by lowering ambient pH in these mutants (Rollins and Dickman, 2001), suggesting a more complex role than that of pH alone. Nevertheless, the *S. sclerotiorum pac1* gene, a component of a regulatory pathway mediating pH regulated gene expression is required for growth at neutral pH, full virulence, normal oxalic acid production and sclerotial development which clearly demonstrates that pH responsive gene expression is an important aspect in *S. sclerotiorum* development and pathogenesis (Rollins, 2003).

Rollins and Dickman (1998) found that cyclic AMP (cAMP) plays a role in the early transition between mycelial growth and sclerotial development. An increase in endogenous or exogenous cAMP level inhibits sclerotial development but elevates oxalic acid accumulation in *S. sclerotiorum*. Increase in cAMP levels inhibits *Smk1*, a mitogen activated protein kinase (MAPK) in *S. sclerotiorum* which is also shown to be necessary for sclerotiogenesis (Fig. 8.1.1). Interestingly, *Smk1* transcripts are maximally expressed under acidic conditions, implicating MAPK cascades via pH and cAMP dependent signaling as regulatory elements to sclerotial development (Chen et al., 2004). To examine further the influence of cAMP pathways on sclerotiogenesis, Harel et al. (2005) demonstrated that cAMP dependent protein kinase A (PKA) levels increase during sclerotial development in wild type strains but stay at low levels in mutants unable to produce sclerotia. Furthermore, applications of environment cues that increase PKA levels are correlated with the production of sclerotia like precursors in the non sclerotium producing mutants (Fig. 8.1.1). However, knock out mutant in the PKA catalytic subunit gene *pka1* show sclerotial development. cAMP responsiveness and pathogenicity similar to wild type, suggesting a PKA independent pathway or additional PKA encoding genes involved in sclerotiogenesis (Jurick et al., 2004). Chen and Dickman (2005) further suggest that sclerotial inhibition by cAMP is PKA independent as inhibition of PKA activity by specific PKA inhibitors has no effect on cAMP dependent MAPK inhibition or sclerotial development.

Model of Rasp-1 mediated mitogen activated protein kinase (MAPK) inhibition by cyclic AMP (cAMP) in *Sclerotinia sclerotiorum*. The schematic shows inhibition

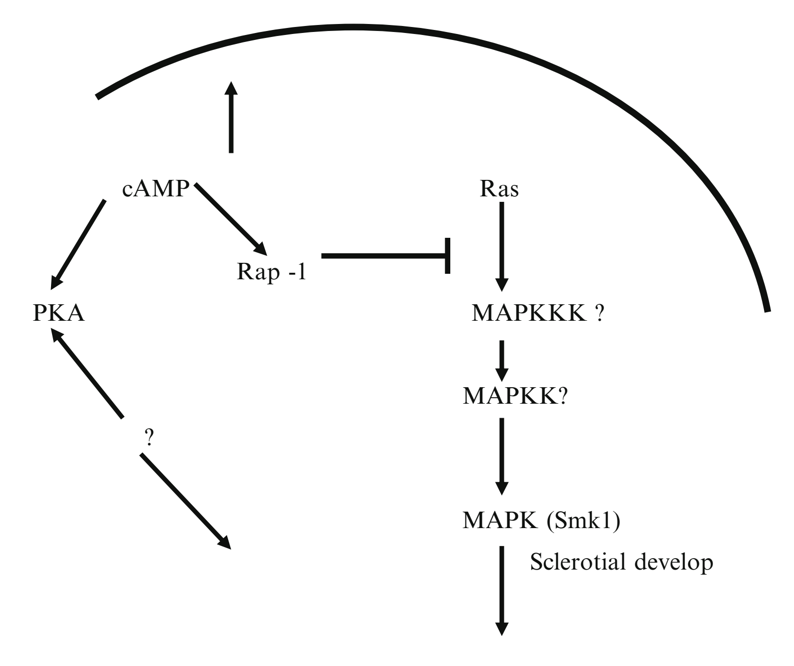


Fig. 8.1.1 Model of Rasp-1 (Adapted from the publication of Chen and Dickman, 2005. With permission)

by cAMP/rap-1 in Ras/ MaPK signaling pathway and the influence of protein kinase A (PKA) on sclerotial development. PKA levels increase during sclerotial development. Conversely, application of environment cues that increase PKA levels correlate with production of sclerotia like precursors in non-sclerotium producing mutants (Harel et al., 2005). Chen and Dickman (2005) show that Rap-1 is activated by cAMP and presumably binds to unidentified upstream MAPK kinase (MAPKKK) and MAPK kinase (MAPKK) components in the Ras/MAPK pathway. This binding leads to inactivation of a downstream MAPK (*Smk1*) which thus suppresses sclerotial development.

Furthermore, the small GTPase Ras an upstream activator of the MAPK pathway is shown to be necessary for normal sclerotial development since loss of Ras activity blocked MAPK activation, suggesting that a conserved Ras/MAPK pathway is required for sclerotiogenesis (Fig. 8.1.1). Interestingly, inhibition of Rap-1 a member of the Ras family of protein restore MAPK activation and sclerotial development normally block in the presence of cAMP. Taken together, these results suggest that sclerotial development relies on a mechanism requiring the involvement of the Ras/MAPK pathway that is negatively regulated by Rap-1 in a PKA independent cAMP signaling pathway (Fig. 8.1.1). These studies illustrate a finally orchestrated interplay between cAMP signaling and ambient pH sensing in sclerotiogenesis where the absolute role of PKA is currently unclear.

8.2 Sclerotium Formation

Sclerotia of *S. sclerotiorum* and related species form readily and are very conspicuous (Willetts and Wong, 1980). Details of sclerotium development have been described by several workers (Chet and Henis, 1975; LeTourneau, 1979; Saito, 1977; Willetts and Wong, 1980). Four phases of sclerotium formation are recognized (LeTourneau, 1979; Saito, 1977; Trevethick and Cooke, 1973; Willetts and Wong, 1980) (Fig. 8.2.1; Plate 8.2.1).

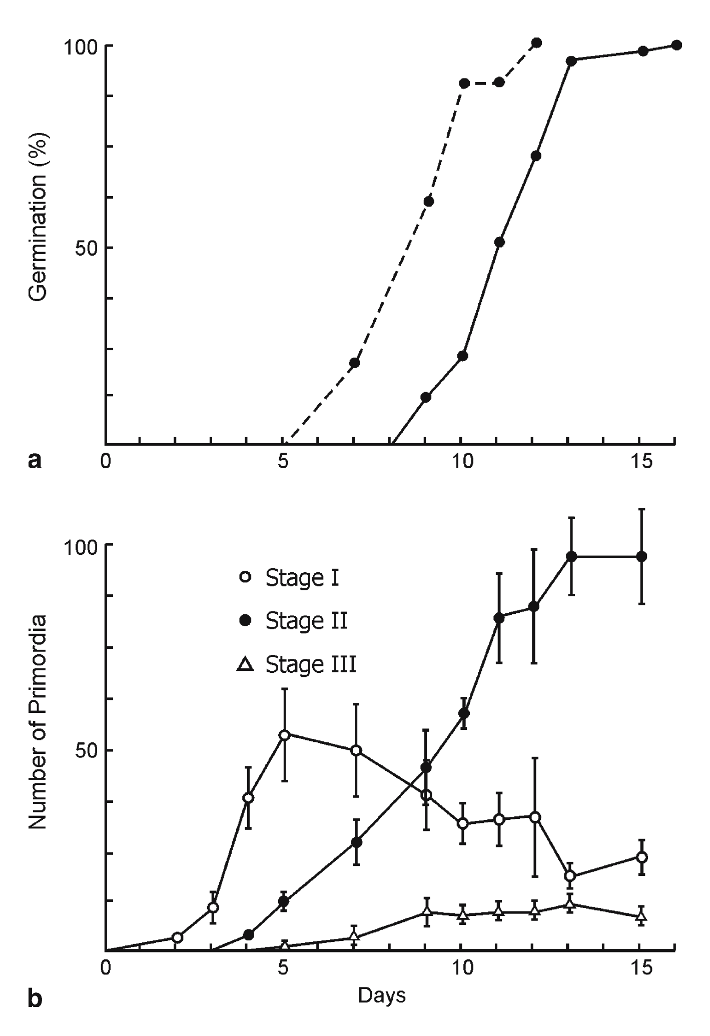


Fig. 8.2.1 Comparative time requirement for sclerotial germination and for stipe primordium formation in sclerotial tissue; (A) Germination rates of sclerotia (solid line) and formation of stage IV primordia in sclerotia (dotted line); (B) Number of the stipe primordia in the developmental stages (Adapted from the publication of Saito, 1977. With permission)

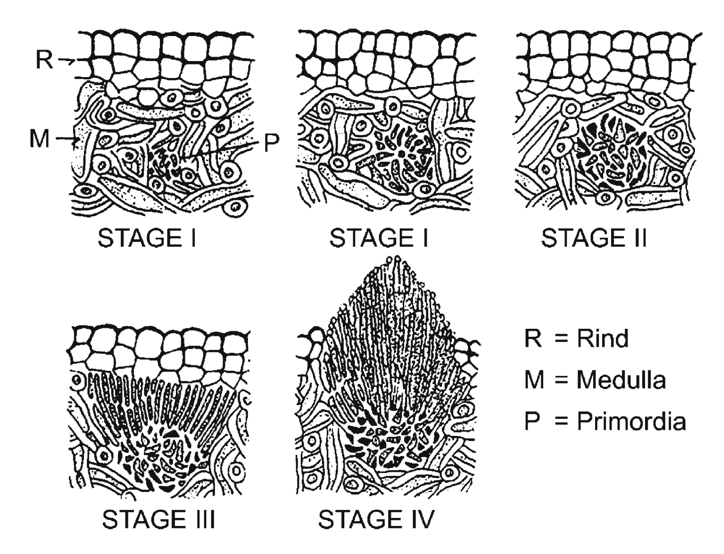


Plate 8.2.1 Diagram illustrating a developmental sequence of apothecial stipe primordium (Adapted from the publication of Saito, 1977. With permission)

1. Initiation, which involves the formation of discrete small sclerotial primordia on the mycelium (Stage I)
2. Growth, in which the primordia rapidly develop into white compact hyphal masses and attain a maximum size (Stage II)
3. Maturation, which sets in as the sclerotia cease to grow in size and is characterized by internal changes, dehydration and pigmentation (Stage III)
4. Primordia rupture the rind and begin to grow as young apothecial stipes (Stage IV)

When mature, sclerotia function as “resting structures” in the life cycle of this pathogen and are able to resist adverse environmental conditions (Adams and Ayers, 1979; Schwartz and Steadman, 1978; Willetts and Wong, 1980). Much of our knowledge of the effects of environmental variables on sclerotium formation comes from studies done in the laboratory. Results from some of these studies have been positively correlated with observations in the field, but others have not been verified in this way. Sclerotium initials are formed as a response to the production of specific compounds by the mycelium or when the mycelium reaches a particular physiological state (Travethick and Cooke, 1973). When sclerotium initials are formed, they either develop fully or not at all. Sclerotia rarely if ever, remain in a partly mature state. Whether growth or maturation takes place depends largely on the nutritional status of the medium, whereas sclerotium initiation is more dependent on non-nutritional factors (Trevethick and Cooke, 1973).

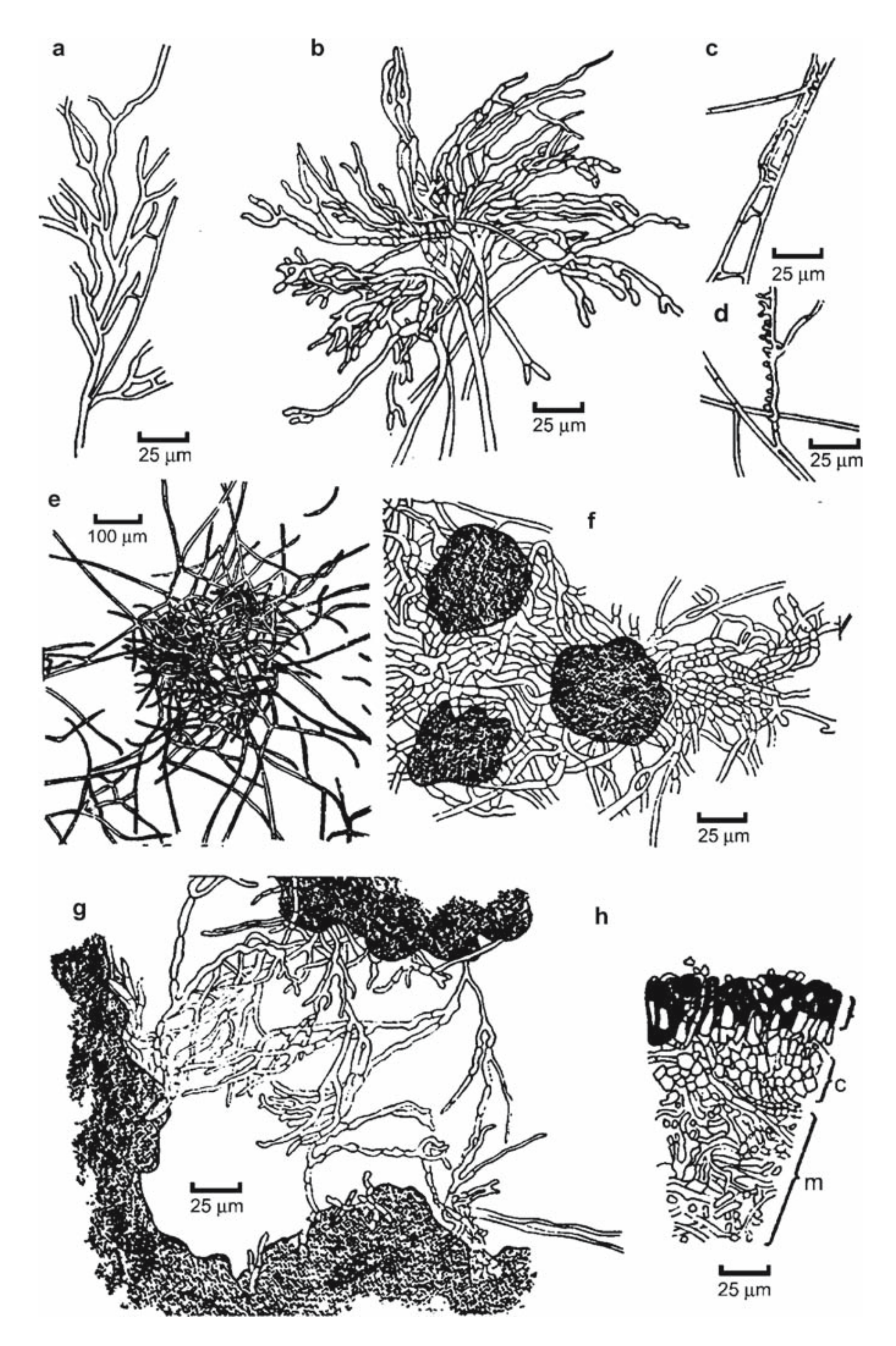
8.3 Cytology and Morphology of Sclerotia

Although there are variations due to isolates, substrates and conditions but sclerotium formation in *S. sclerotiorum* follows a general sequence. When ascospores, mycelia or sclerotia are placed on a solid nutrient medium, hyphae grow out to form a thin film of growth over the surface. When the colony reaches the edge of the container or when growth is otherwise restricted, the mycelium mat thickens and produces white mounds of mycelium covered with small liquid droplets. As sclerotium increases in size, the surface begins to darken and larger exudate droplets are apparent. As the surfaces of the sclerotium continue to darken until it is black, the exudates droplets disappear. Within approximately one week or so, the process is complete and a mature sclerotium can be removed from the substrate. Sclerotia often form at the edge of the plate but sometimes the sclerotia cover the substrate in a series of concentric circles or some other regular pattern. These patterns appear to be due to endogenous rhythms in some isolates of the fungus (Humpherson-Jones and Cooke, 1977b). Details of sclerotium formation have been studied by light transmission and scanning electron microscopy (Colotelo, 1974; Saito, 1974b, 1977; Willetts and Wong, 1971). The vegetative hyphae contain various organelles and a simple, single perforated septum characteristic of other Ascomycotina. Saito (1974a) also noted a lomasome-like structure between the plasma membrane and the one-layered cell wall and an unidentified tubular vesicle complex in the cytoplasm.

Sclerotium development in several *Sclerotinia* spp. has been studied by Willetts and Wong (1971). In large sclerotia types (designated as *S. sclerotiorum* and *S. trifoliorum),* sclerotium development is of the terminal type. Initials arise from anastomoses of long primary hypha and several initials fuse to form a large sclerotium. Small sclerotia types (designated as *S. libertiana* and *S. minor*) form initials by a special type of strand development. Initials arise in a position laterally to the main hyphae from short, aerial hyphae which become interwoven (Plates 8.3.1–8.3.3). With continued growth, the internal (medullary) cells become compact and rind cells begin to form beneath the surface mycelial network. During this period, intercellular spaces fill with liquid which is continuous with the surface droplets (Colotelo, 1974). Cells of the developing sclerotia contain numerous ribosomes and multivesicular and multitubular lomasomes. Invaginations of the plasma membrane are noted frequently. As the sclerotium matures, the original chitinous wall of the internal hyphae becomes covered with a thick fibrillar layer. Histochemical tests in combination with enzyme treatments indicate that this layer is composed of β 1–3 glucan and protein (Saito, 1974a, 1977). These same hyphae contain a polysaccharide,

→

Plate 8.3.1 Large type sclerotium *Sclerotinia sclerotiorum* and *S. trifoliorum*. (a) Normal mycelium growth; (b) Early stage in development of sclerotial initials, apical growth has been arrested and numerous dichotomous branches have been developed; (c) Anastomoses of adjacent hyphae; (d) Numerous protuberances of the type associated with anastomoses; (e) The tufted appearance of a developing sclerotium. At this stage the hyphae are white; (f) Three small pigmented initials with



interweaving of hyphae in the area between them; (g) Two large pigmented initials joined by hyphae. The space between the initials becomes filled with mycelium and possibly the initials will coalesce to form a large sclerotium; (h) Part of transverse section of periphery of mature sclerotium of *S. sclerotiorum* to show rind; (i) Of thick walled cells, cortex; c: of hyphae pseudoparenchymatous cells and medulla; m: of irregularly arranged hyphae (Adapted from the publication of Willetts and Wong, 1971. With permission)

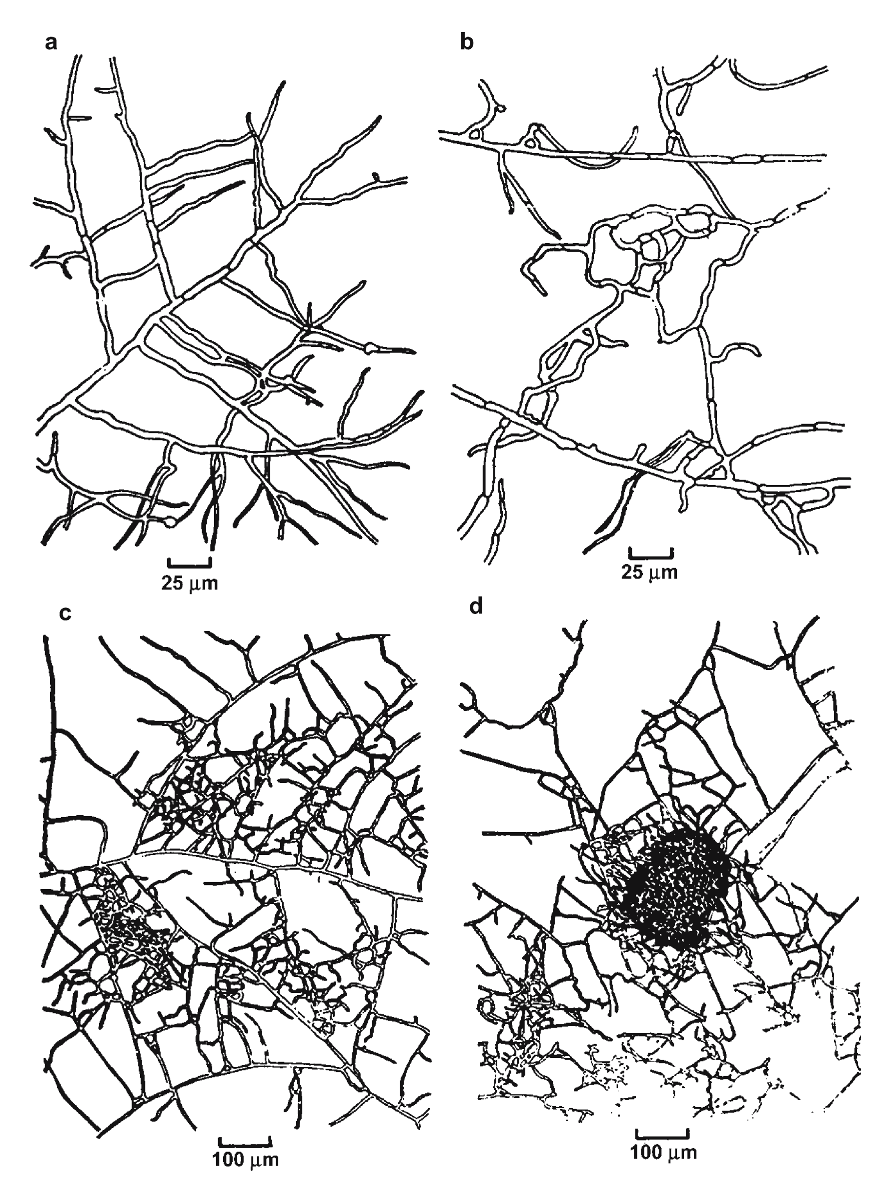


Plate 8.3.2 Small type sclerotium of *Sclerotinia minor* and *Sclerotinia libertiana*. (a) Normal mycelium growth; (b) Very early stage in development of sclerotial initials, several branches have anastomosed; (c) Later stage in formation of initials; (d) Small differentiating sclerotium (Adapted from the publication of Willetts and Wong, 1971. With permission)

probably glycogen, within the cells. The cristae of the mitochondria become indistinct and electron dense materials, possibly polyphosphates, accumulate in the vacuoles as the sclerotia mature. Mature rind cells contain melanin and have numerous pores or openings (Jones, 1970; Saito, 1977). When mature, a sclerotium consists

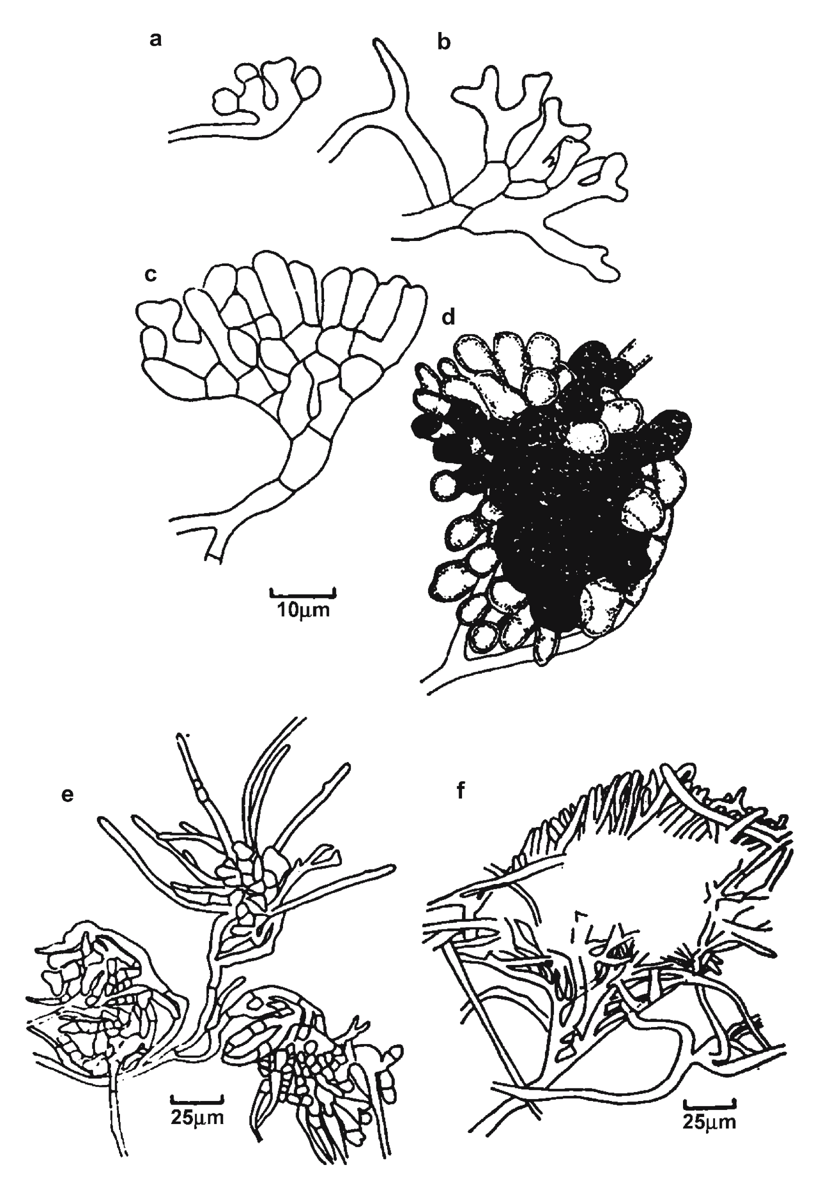


Plate 8.3.3 (a–c) Stages in the development of a small hyphal aggregate of *Sclerotinia minor* by dichotomous branching and septation. (d) Final stage in development. Most of the cells have become pigmented. (e) Small spore like masses. Vegetative hyphae have grown out from some of the cells. (f) A hyphal mass that formed on the surface of cellophane placed over the culture medium (Adapted from the publication of Willetts and Wong, 1971. With permission)

of a black rind approximately three cells wide and a medulla of prosenchymatous tissues embedded in a fibrillar matrix (Colotelo, 1974; Saito, 1974a). Kosasih and Willetts (1975) have described a cortical layer two to four cells thick between the rind and the medulla.

8.4 Composition of Sclerotia

Proximate chemical analysis of sclerotia collected from commercial bean and pea cleaning operations showed less than 2 per cent crude fat (diethyl ether extract), 3.5–5.0 per cent ash and 20–25 per cent protein (total Kjeldahl N × 6.25). A considerable proportion (20–25 per cent) of the dried ground sclerotia is soluble in ethanol. A protein content of 10–15 per cent is obtained if the calculation is based on alcohol-insoluble nitrogen. Sclerotia grown on a synthetic agar medium contain less crude fat, alcohol soluble material and protein than do sclerotia from field collections. Other analyses of the component hydrocarbon, free fatty acids and amino acids in various factions of such sclerotia are available (LeTourneau, 1979; Weete et al., 1970). Sclerotia accumulate large amounts of unsaturated fatty acids (oleic and linoleic) for new fungal growth (Khalil and Rogab, 1990).

Carbohydrates make up the greatest portion of the dry matter of mature sclerotia. Chemical analysis of sclerotia indicates about 25 per cent β-glucan (LeTourneau, 1979). Histochemical evidence (Saito, 1974a, 1977) shows the presence of polysaccharides, chitin and glycogen. Infrared spectra studies do not indicate the presence of cellulose (Michell and Scurfield, 1967). Trehalose, a non reducing disaccharide and mannitol (a sugar alcohol) are the major low molecular weight carbohydrates found in sclerotia. Monosaccharides, such as glucose or fructose usually are present in the mycelia and sclerotia. The occurrence of other sugars and sugar alcohols is dependent on the carbon source in the medium (Wang and LeTourneau, 1971).

8.5 Metabolites Associated with Sclerotium Formation

The formation of sclerotia entails extensive cellular changes and the mobilization and deposition of many materials. The factors involved in the initiation phase have received little attention. As pointed out by Willetts (1978), vegetative hyphae usually grow away from one another. In the formation of initials, there must be an attraction so that hyphal fusions occur. Because initials usually form after growth has covered the substrate, their formation may be a response to changes in nutrient availability. The fact that several fungi, including *S. sclerotiorum*, form sclerotia when mycelial mats are transferred to a medium low in nutrients supports this hypothesis (Christias and Lockwood, 1973). Initials may form only after certain metabolic products are produced in the mycelium or in the medium. Organic acids, long known to be produced by *Sclerotinia* spp. may play a role in sclerotium formation (Humpherson-Jones and Cooke, 1977c; Wang and Le Tourneau, 1971). While the amount and kinds of organic acids vary with the isolate and the medium, oxalic, fumaric, malic and succinic acids usually are produced by *S. scleroiorum* (Corsini and Le Tourneau, 1973; Humpherson-Jones and Cooke, 1977c). Other acids e.g., citric, glycolic, glyoxylic and a-ketoglutaric also have been detected (Colotello, 1973; Humpherson-Jones and Cooke, 1977c). Enzymes for the production of Krebs

cycle acids and oxalic acid are present in *S. sclerotiorum* (Corsini and Le Tourneau, 1973; Maxwell, 1973).

When *S. sclerotiorum* is grown on a liquid medium, the pH of the culture filtrate drops to pH 3–4 and then tends to increase slightly (Wang and LeTourneau, 1971). The drop in pH occurs at a time of maximum acid production and when the specific activity of Krebs cycle enzymes is high. Numerous sclerotia form when the fungus grows on a medium which supports good growth and the initiation of sclerotia tends to coincide with the pH drop. When the fungus is grown on a medium which supports very little growth or sclerotium formation, the pH of the culture filtrate usually increases (Wang and LeTourneau, 1971). The addition of oxalic and Krebs cycle acids to the medium does not enhance sclerotium formation (Humpherson-Jones and Cooke, 1977c; Wang and LeTourneau, 1971). Once initials have formed, materials must be supplied for the synthesis of reserves and other compounds that are present in the mature sclerotium. Studies on the translocation of solutes have been summarized by Chet and Henis (1973) and Willetts (1978).

Developing sclerotia are covered with droplets that are continuous with the interior of the developing sclerotium (Colotelo, 1974). These droplets are covered by a membrane and contain a variety of inorganic and organic materials, including several enzymes, which change in concentration as the sclerotium matures (Colotelo, 1973). The precise role of the exudate is still open to interpretation. It may play an osmotic role in translocation or it may be involved in the loss of water from the sclerotium during maturation (Willetts, 1978).

Although many enzymes have been detected in sclerotia, it is not yet clear how the activity of these enzymes is controlled or coordinated or which enzymes may play key roles in morphogenesis. From results of polyacrylamide-gel electrophoresis studies, Wong and Willetts (1974) suggested that suppression of glycolysis, the Krebs cycle and stimulation of the pentose phosphate pathway are involved during the compaction and maturation of sclerotia. Various enzymes of phenol oxidation have been detected (Colotelo, 1973; Jones, 1970; Wong and Willetts, 1974). Tyrosinase may be involved in the initiation of sclerotia and other phenol oxidases may be involved in the formation of the darkened rind (Wong and Willetts, 1974).

8.6 Factors Affecting Sclerotium Formation

There are number of factors which influence formation of sclerotia *in vitro* and/or *in vivo* and/or both.

8.6.1 Effect of Temperature

Sudden changes in temperature have not been shown to stimulate the formation of sclerotium initials in either *S. sclerotiorum* or *S. minor* (Willetts and Wong, 1980).

S. sclerotiorum in culture produces sclerotia over a range of temperatures from 0°C to 30°C (Bedi, 1962; Marukawa et al., 1975a; LeTourneau, 1979) and although the fungus does grow slowly at temperatures below 5°C, no sclerotia are produced (Bedi, 1962). The most sclerotia are produced at about 15°C (Bedi, 1962). Fewer but larger sclerotia are produced at lower temperatures (Bedi, 1962; Marukawa et al., 1975a; LeTourneau, 1979) and the smallest sclerotia are produced at the highest temperature (Bedi, 1962). Observations in the field have shown that more sclerotia are produced at about 17°C than at temperatures below 10°C (Kruger, 1975a). It is interesting that the optimum temperature recorded for sclerotium formation (about 15°C) is much lower than the optimum temperature for mycelium growth i.e., about 20–25°C (Bedi, 1962; Willetts and Wong, 1980). This is likely a response of this pathogen to the environment in that resistant propagules are formed when environmental conditions become unfavourable for mycelial growth.

8.6.2 Effect of Light

Results of studies on the effects of light on sclerotium formation of *Sclerotinia* spp. are conflicting (LeTourneau, 1979). In general, more sclerotia are produced in the light than in the dark (LeTourneau, 1979; Trevethick and Cooke, 1973), although those produced in the dark are larger than those produced in the light (LeTourneau, 1979). Light is most effective in increasing the number of sclerotia when the mycelium is illuminated before sclerotium initials are formed (Humpherson-Jones and Cooke, 1977a; LeTourneau, 1979). More sclerotia are produced as light intensities increase (Humpherson-Jones and Cooke, 1977a; Trevethick and Cooke, 1975a), but this increase in number is reflected by a decrease in the size of individual sclerotia so that the total yield of sclerotium material remains the same (Humpherson-Jones and Cooke, 1977a). No further increase in number of sclerotia occurs at light intensities above 30 lumens/ft^2 (Trevethick and Cooke, 1973). The number of sclerotial primordia and mature sclerotia increases with increasing light intensity up to about 500 lux, but dry weight of mature sclerotia decreases. Short light periods (48 h) up to 5,000 lux increase both primordia and mature sclerotia (Kim, 1976).

The effect of light appears to be through the direct induction of sclerotium initials on undifferentiated hyphae (Humpherson-Jones and Cooke, 1977a). Sudden changes in light intensity do not seem to stimulate the formation of sclerotium initials (Willetts and Wong, 1980), although the number and size of sclerotia that will form on a culture can be determined by the length of the photoperiod (Trevethick and Cooke, 1971). Sclerotium formation is most vigorous when mycelia are exposed to wavelengths in the blue to ultraviolet range of the spectrum; red, infra-red or green light do not seem to have any stimulatory effect (Humpherson-Jones and Cooke, 1977a; LeTourneau, 1979). However, irradiation with UV has no effect on the production of sclerotia but red and blue light produces, a few, but large sclerotia in culture (Nagy and Fischi, 2002).

8.6.3 Effect of Nutrients

As with temperature and light, sudden changes in nutrient levels have not been found to stimulate the formation of sclerotium initials (Willetts and Wong, 1980). However, sclerotium growth and maturation is dependent on the type and amount of nutrients present (Grogan, 1979; Trevethick and Cooke, 1973). Zinc is essential for sclerotium formation (LeTourneau, 1979; Trevethick and Cooke, 1971; Vega and Le Tourneau, 1974) but it is known also to be an essential element for the growth of most fungi (Vega and Le Tourneau, 1974). Potassium, phosphorus, magnesium and sulfur also appear to be essential requirements (LeTourneau, 1979; Purdy and Grogan, 1954).

Sclerotinia spp. utilizes several organic compounds as sources of carbon for both mycelial growth and sclerotium formation (LeTourneau, 1979; Wang and LeTourneau, 1971). Both organic and inorganic compounds are used as sources of nitrogen and there is evidence that sclerotium formation is affected by both the form of nitrogen and the carbon/nitrogen ratio (LeTourneau, 1979; Marukawa et al., 1975a). Growth and sclerotium formation of *S. sclerotiorum* occurs only when the inorganic macronutrients, P, K, Mg and S are present in the medium and is enhanced by the addition of an inorganic micronutrient mixture (Purdy and Grogan, 1954). When the fungus is grown in a liquid medium containing purified chemicals in plastic flasks, less dry weight is produced and sclerotia do not form in the absence of Zn (Vega and Le Tourneau, 1974). Sulfur-containing amino acids, metabolic inhibitors and chelating compounds indirectly affect sclerotium formation by altering unrelated metabolic processes, and these changes are reflected in the number of sclerotia produced (Trevethick and Cooke, 1971). In general as more nutrients become available, more sclerotia are produced (Trevethick and Cooke, 1973).

8.6.4 Effect of pH and Osmotic Potential

Most workers have found that *S. sclerotiorum* can grow and produce sclerotia on media with an initial pH in the range of 2.5–9.0 (Marukawa et al., 1975a). The results of such studies undoubtedly depend on the type of medium, especially the buffering capacity. The pH of the medium may be changed during growth by the production of organic acids (Corsini and Le Tourneau, 1973). When salts or sucrose are added to an agar medium, mycelial growth of *S. sclerotiorum* is stimulated by decreasing the osmotic potential from −1 to −14 bars. Growth decreases below this value, but there is measurable growth at −100 bars. Sclerotia are formed at −65 bars but not at −73 bars (Grogan and Abawi, 1975).

8.6.5 Effect of Specific Compounds

Some isolates of *Sclerotinia* spp. lose the ability to produce sclerotia after repeated sub-culturing (Marukawa and Satamura, 1977). This may be due to the inability of

the fungus to synthesize specific compounds required for sclerotium formation. Several investigators have attempted to isolate such compounds. In such studies sclerotium production (number or weight) is measured after old culture filtrates (staling products) are added to a fresh medium, with such a bioassay, it is possible to fractionate active preparations. Humpherson-Jones and Cooke (1977c) showed that two unidentified acids enhance sclerotium production. Marukawa and Satamura (1977) suggested that sclerin, a known metabolite of *Sclerotinia* spp. in combination with other compounds, such as phenols is involved in melanogenesis and formation of hyphal aggregates (Marukawa et al., 1975b).

8.6.6 Effect of Inhibitors

The failure of some isolates of *Sclerotinia* spp. to produce sclerotia might be due to the production of inhibitors. Some compounds and the approximate concentration required to inhibit the formation of sclerotia are p-aminobenzoic acid, 6 mM (Marukawa et al., 1975b); phenylthiourea, 1 mM (LeTourneau, 1976); Al^{+++}, 0.6 mM (Orellana et al., 1975) and fluorophenylalanine, 0.1 mM (Dimopoulou, 1975). It is of interest that p-aminobenzoic acid and phenylthiourea are known inhibitors of polyphenyloxidase. Further study is required to find more potent inhibitors and to determine the mode of action of these inhibitors.

8.6.7 Effect of Soil and Host Residues

Many other factors are known to influence sclerotium formation by *S. sclerotiorum* not only in host plants, but also in the soil after harvest residues are ploughed under. Harvest residues have a stimulatory effect and increase the density of sclerotia in the soil (Kruger, 1975b). Soil type also appears to have an effect (Kruger, 1975b). More sclerotia are produced when soils are relatively damp to wet (Kruger, 1975a), although this is likely due to the availability of water-soluble nutrients. The presence of other soil micro-organisms also affects sclerotium formation, but the response of *S. sclerotiorum* to these other microbes is largely dependent on soil temperature (Bedi, 1958).

8.7 Sclerotium Survival

Sclerotia are the primary survival structures of *Sclerotinia* spp. (Adams and Ayers, 1979; Coley-Smith and Cooke, 1971; Cooke et al., 1975; Schwartz and Steadman, 1978; Willetts and Wong, 1980), capable of maintaining the fungus in a dormant state and germinating when environmental conditions favour its growth (Coley-Smith

and Cooke, 1971). Large numbers of sclerotia are produced during epidemics (Grogan, 1979), but since soil populations of sclerotia remain relatively stable, it appears that the actual percentage that survive is low (Abawi and Grogan, 1979; Grogan, 1979). In contrast, soil populations do not decrease for several years when non-host crops are grown, indicating that some sclerotia are long-lived (Morrall and Dueck, 1982; Schwartz and Steadman, 1978).

The longevity of sclerotia is difficult to measure in the field even in the absence of susceptible hosts because the "primary" sclerotia produce "secondary" or "daughter" sclerotia in the absence of suitable substrates for colonization (Adams and Ayers, 1979; Coley-Smith and Cooke, 1971). This not only causes an increase in the number of sclerotia, but it also extends their viability (Coley-Smith and Cooke, 1971; Kruger, 1975b; Willetts and Wong, 1980). Data on the survival of sclerotia in the field is quite variable with estimates of their longevity ranging from a few weeks to over ten years (Adams and Ayers, 1979; Coley-Smith and Cooke, 1971; Cooke et al., 1975; Grogan, 1979). For sclerotia of *S. trifoliorum*, Dillon-Weston et al. (1946) estimated that sclerotia can survive for six to eight years. Tribe (1957) reported that *S. trifoliorum* sclerotia buried at a depth greater than 6.4 cm persist for about eight years. Davis (1925) is of the opinion that sclerotia of *S. sclerotiorum* near the soil surface do not remain viable for more than one year. Young and Morris (1927) however, reported that at least a four year rotation is essential before sunflower can be grown on a field with a history of *Sclerotinia* wilt. In the case of bean white mould, Starr et al. (1953) suggested a three to five year period of non-host crops. Cooke (1973) reported that rotating beans with corn and sugar beets every third year is not an effective control practice in Nebraska. Brown and Butler (1936) reported that under favourably dry conditions, sclerotia of *S. sclerotiorum* remain viable for at least ten years. However, Quentin (2004) reported that *S. sclerotiorum* can survive for up to ten years in the soil, benefiting from irrigation and warmers climates. This is in contrast to the estimated four to five years of survival for sclerotia in field soil. Observational errors alone cannot explain this variability, but differences in environmental conditions between localities may account for much of it since environmental factors do influence sclerotium survival (Adams and Ayers, 1979; Coley-Smith and Cooke, 1971; Willetts and Wong, 1980). It is also important to recognize that environmental variables are usually complementary in their action, a change in one variable leading to change in others (Bedi, 1961; Coley-Smith and Cooke, 1971).

The following factors affect the survival of sclerotia of *Sclerotinia* spp. under field conditions.

8.7.1 Effect of Soil Moisture, Texture, pH, Temperature, Nutritional Status and Depth of Sclerotial Burial in the Soil

Sclerotia survive best in dry soils and poorest in wet soils (Coley-Smith and Cooke, 1971; Imolehin and Grogan, 1980). Pollution of surface irrigation water

during irrigation runoff from agricultural land by sclerotia of *S. sclerotiorum* causing bean white mould has been reported (Steadman et al., 1975). Sclerotial bodies remain viable for at least 10–21 days in flowing water and can be potential inoculum for white mould disease in beans, because they lack the ability to regulate water loss. Sclerotium survival during dry periods is due to the innate resistance of individual sclerotial cells to desiccation, not to the physical properties of the outer tissues of the sclerotium (Trevethick and Cooke, 1973). Increasing soil moisture accelerates sclerotium degradation (Coley-Smith and Cooke, 1971) and also increases their susceptibility to damage from solar radiation (Willetts and Wong, 1980), although the latter is probably of minor importance in most field situations (Willetts and Wong, 1980). Soil temperatures of 35°C and higher for several weeks reduces sclerotium survival, but this is also of minor importance since high soil temperatures for these extended periods do not occur under natural field conditions in temperate regions (Adams, 1975; Adams and Ayers, 1979). However, the combined effects of both high soil moisture and temperature drastically increase the level of sclerotium destruction (Cooke et al., 1975; Willetts and Wong, 1980). Moore (1949) reported that nearly 100 per cent of the sclerotia are killed when soil is flooded with water for 16–31 days. Flooding has been used in some localities to eliminate sclerotia from fields since the high moisture levels promote sclerotium decay (Adams and Ayers, 1979; Coley-Smith and Cooke, 1971; Roten and Palti, 1969; Steadman, 1979), but since large volumes of water are required, this technique has limited usefulness in non-irrigated areas (Steadman, 1979).

Burial of sclerotia at 4 cm depth for 35 weeks reduces recovery of sclerotia to zero in sandy clay loam and by 50 per cent in sandy clay. At the soil surface, recovery is reduced by 55 per cent in sandy clay loam and by 10 per cent in sandy loam. Less than 50 per cent of sclerotia recovered are viable (Merriman, 1976). However, according to Adams (1975), sclerotia of *S. sclerotiorum* buried in soil survived well over 15 months at 1–12 in. depth, but poorly at 25 in. Nearly 75 per cent of the sclerotia recovered after three years buried at 5, 12, 15, and 20 cm below the soil surface germinate and form apothecia in culture (Cooke et al., 1975). Sclerotia of *S. sclerotiorum* remain viable at a soil depth of 20 cm for >18 months, whereas none of those in the surface germinated after six months under Indian conditions. Apothecia formed between November and March produce ascospores which are the primary source of infection of cauliflower (Sharma and Sharma, 1986a). Sclerotia of *S. minor* survive better at shallower depth in soil (Fig. 8.7.1.1) than at greater depths (Imolehin and Grogan, 1980). According to Kakoti and Saikia (1997), sclerotia survive on the soil surface for up to seven months, eight months at 5 cm depth, seven and six months when buried at 10 and 15 cm depth respectively. Sclerotia buried at 20 cm depth shows decline in viability within five months. The depth at which sclerotia are buried in the soil affects the degree and frequency of drying (Willetts and Wong, 1980). The drying and wetting of sclerotia causes them to leak nutrients, which affects their survival by decreasing their nutrient reserves and by stimulating other soil microorganisms to colonize them (Adams, 1975; Willetts and Wong, 1980).

Sclerotia survive for more than ten years under dry conditions. Sclerotia distributed in field soil at depths of 5 mm and 10–20 mm survives for four and five years respectively under Tokachi, Japan conditions (Akai, 1981). Flooding of fields for two to three weeks with a history of lettuce drop caused by *S. minor* and *S. sclerotiorum* reduces population of viable sclerotia (Matheron and Porchas, 2005).

The nutrient status of the soil itself is also important in determining sclerotium survival (Coley-Smith and Cooke, 1971). Nutrient-rich soils have large populations of many different micro-organisms in them that decompose organic matter, so any sclerotia in these soils are also susceptible to microbial breakdown. Soil pH, on the other hand, does not seem to be very important in determining sclerotium survival (Adams and Ayers, 1979; Coley-Smith and Cooke, 1971).

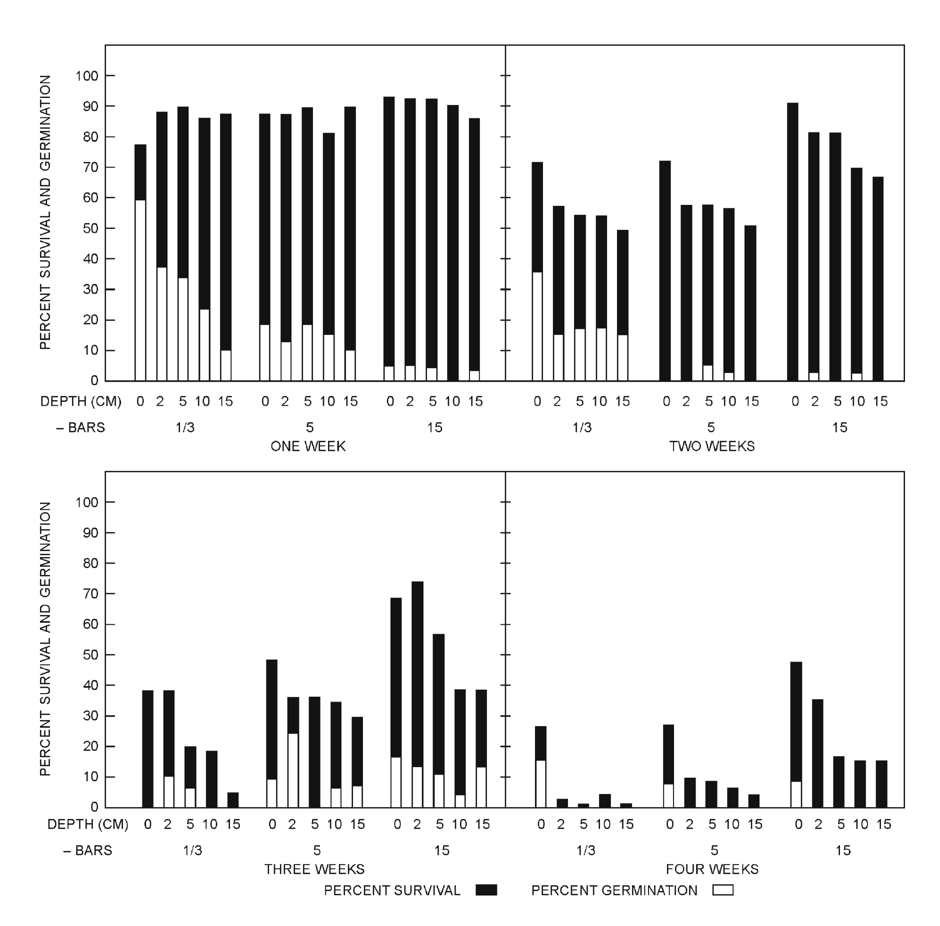


Fig. 8.7.1.1 Effect of depth of burial and soil moisture tension on survival and germination of sclerotia of *Sclerotinia minor* (Adapted from the publication of Imolehin and Grogan, 1980. With permission)

8.7.2 Effect of Other Soil Micro-organisms

The biological component of soil is probably the most important factor in determining sclerotium survival (Adams and Ayers, 1979). Several microbes are known to be antagonists or mycoparasites of *Sclerotinia* spp. sclerotia (Table 19.9.1). Of these species, *Coniothyrium minitans*, *Sporidesmium sclerotivorum* and *Trichoderma* spp. are probably the most important under natural conditions (Adams and Ayers, 1979; Coley-Smith and Cooke, 1971; Dueck, 1977; Huang, 1976, 1977; Tribe, 1957; Willetts and Wong, 1980). More details on parasitism are given in the biological control section (19.9, 19.10).

The variation in the recorded longevity of *Sclerotinia* is at least partly due to differences between localities in the population of soil micro-organisms (Huang, 1977; Willetts and Wong, 1980). Soil moisture, pH, temperature and the nutrient status of the soil indirectly affect the survival by influencing the type and number of soil microorganisms that are present in a given locality (Steadman, 1979), as well as their activity in terms of the breakdown of organic matter in the soil. The presence of soil nutrients also stimulates dormant sclerotia to germinate, which increases their susceptibility to microbial attack (Coley-Smith and Cooke, 1971). Damage to the sclerotial rind also increases the susceptibility of sclerotia to microbial attack, although rind regeneration can occur (Coley-Smith and Cooke, 1971).

8.7.3 Effect of Animal Feeding

Peanut hay of the exflorunner infested with *S. minor* containing sclerotia was fed for ten days to a crossbred heifer. Viable sclerotia were recovered from fecal and ruminal samples. Survival of sclerotia is greater in ruminal samples than in fecal samples. Cultures of *S. minor* from fecal and ruminal sclerotia are pathogenic to peanut or Tamnutzu under greenhouse conditions (Melouk et al., l989).

8.7.4 Effect of Host Tissues

Sclerotia formed within or outside bean stems were tested by Merriman et al. (1979) for survival when protected or unprotected by bean straw. The most (11 per cent) sclerotia survive when protected in bean straw or when the sclerotia had originated from within stems.

8.7.5 Effect of Soil Atmosphere

Soil atmosphere or aeration appears to influence sclerotium survival, but it is probably mediated via neither germination since oxygen is not likely to be depleted nor

carbon dioxide to accumulate to levels that would persist long enough to be lethal to dormant sclerotia (Coley-Smith and Cooke, 1971).

8.7.6 Effect of Mode of Germination

There does not seem to be any correlation between survival and whether sclerotia germinate myceliogenically or carpogenically (Coley-Smith and Cooke, 1971; Cooke et al., 1975), but sclerotia that germinate myceliogenically also have their longevity reduced, probably because of depleted nutrient reserves (Saito, 1977).

8.8 Sclerotium Dissemination

Sclerotinia spp. become established and is spread from field to field, from one geographical area to another by several means. *Sclerotinia* spp. disseminate from field to field in soil adhering to seedlings, farm equipment, animals or man (Dillon-Weston et al., 1946; Starr et al., 1953) in the form of sclerotia or as mycelium in infected host tissue. On farms where diseased plant tissue is used as cattle feed or bedding, the spreading of manure on fields has been shown (Dillon-Weston et al., 1946) to be a likely means of introducing the pathogen to uncontaminated fields. Brown (1937) showed that less than 2 per cent of the sclerotia of *S. sclerotiorum* fed to sheep passed through the digestive tract in a viable condition. Thus sheep, and possibly other animals, fed diseased plant refuse and then turned out to pasture, can spread the pathogen to *Sclerotinia* free fields. Irrigation also has been shown to be involved in the spread of *Sclerotinia* species from field to field. Steadman et al. (1975) showed that sclerotia of *S. sclerotiorum* can be collected from waterways and in irrigation runoff from fields in Nebraska. Such sclerotia remain viable for at least 10–21 days in flowing water. Patterns of movement of the sclerotia are correlated with previous or current season infection of bean plants. Probably the greatest potential for long distance dissemination of *Sclerotinia* spp. is either by seed infected with mycelia or by seed contaminated with sclerotia. *Sclerotinia* infected or infested seed has been reported for several crops. For details about the seed infection referred to Section 12.6.

8.9 Sclerotia as Inoculum

Very little has been reported on natural populations of sclerotia in soil. Henderson (1962b) reported from 0 to 20 sclerotia of *S. sclerotiorum* per 929 cm^2 (1 ft^2) of field soil to a depth of 5.1 cm. This is the equivalent of zero to three sclerotia per kilogram of soil. Working with sunflower, Hoes and Huang (1975) found approximately two

to three sclerotia of *S. sclerotiorum* per kilogram of non-rhizosphere soil. However, they found about 211 sclerotia per kilogram of soil in the rhizosphere of diseased plants. Abawi and Grogan (1975) determined the inoculum density of *S. sclerotiorum* at various depths in a bean field in New York. At depths of 0–2.5, 2.5–10 and 10–17.5 cm, the number of sclerotia are approximately 7.2 and 0.5 sclerotia per kilogram respectively, before ploughing and near zero at all depths after planting. The inoculum densities of *S. sclerotiorum* in bean fields in western Nebraska are shown to range from 0.1 to 6.2 sclerotia per kilogram of soil (Schwartz and Steadman, 1978). On the basis of this limited amount of information, it appears that the levels of sclerotia in natural soils range from zero to less than ten sclerotia per kilogram in a field ready for planting. According to Adams and Ayers (1979), in three New York States, fields with a history of severe lettuce drop, the inoculum density of *S. minor* ranges from 160 to 820 sclerotia per kilogram of soil. In 11 mineral soil fields in New Jersey, the inoculum density of *S. minor* ranges from 0 to 230 sclerotia per kilogram of soil. In a field with a history of severe *Sclerotinia* peanut blight in Virginia, the inoculum density ranged from 35 to 100 sclerotia per kilogram of soil. Thus, in fields with a history of losses due to *Sclerotinia* spp. the inoculum density of *S. minor* has been found to be 10 to 100 times greater than that of *S. sclerotiorum*.

The sclerotial inoculum density of *Sclerotinia* species can be increased in two ways:

(i) By the production of secondary sclerotia in soil
(ii) By the production of sclerotia on their hosts

Williams and Western (1965b) have shown that sclerotia of *S. sclerotiorum* and *S. trifoliorum* are capable of forming secondary sclerotia in soil in the absence of a host. Although these two species as well as *S. minor* can form secondary sclerotia and thereby increase their numbers.

The importance of the host in the production of sclerotia has been determined by Stevens (1911), who reported that if a plant bed 2.7 × 61 m (9 × 200 ft) containing 2,000 lettuce plants is diseased, as many as 17,000 sclerotia of *S. minor* can be produced and eventually worked into the soil. If thoroughly incorporated into the soil to a depth of 15 cm, this will increase the inoculum density by about 0.5 sclerotia per kilogram of soil. Adams (1975) reported that as many as 1,000 sclerotia of *S. minor* formed on a single, diseased, romaine lettuce plant. Assuming that each plant occupied 929 cm^2 (1 ft^2) of a field and each plant produced 1,000 sclerotia, the inoculum density of the field would increase by 50 sclerotia per kilogram of soil when the diseased crop is worked into the soil to a depth of 15 cm. Several weed hosts are the significant factor for increasing inoculum densities of *Sclerotinia* spp. under field conditions.

8.10 Sclerotium Germination

Sclerotia of *Sclerotinia* spp. are capable of both myceliogenic and carpogenic germination, producing mycelia and apothecia, respectively (Dueck, 1977; Saito, 1977; Willetts and Wong, 1980). Myceliogenic germination and infection is of

little epidemiological significance for many crops since it occurs only rarely under natural conditions (Grogan, 1979; Abawi and Grogan, 1975, 1979; Akai, 1981), but for other crops it is of major importance (Hoes and Huang, 1976; Huang and Hoes, 1980). Sclerotial mycelium is known to incite infection in rapeseed crops (Dueck, 1977) but the infective potential of sclerotial mycelium is low compared to that of ascospores, which are the primary cause of epidemics (Dueck, 1977; Kruger, 1975a; Scheibert-Bohm et al., 1981; Williams and Stelfox, 1980b). It is possibly that millions of ascospores can be produced from a single apothecium (Abawi and Grogan, 1979; Schwartz and Steadman, 1978; Williams and Stelfox, 1980a). Whereas the sclerotium population in natural soils rarely exceeds ten sclerotia per kilogram of soil (Adams and Ayers, 1979). Furthermore, ascospores are dispersed by air currents for distances of up to several kilometers (Abawi and Grogan, 1979; Williams and Stelfox, 1979) and are thus capable of causing infection far away from the original source of inoculum. However, mycelium from sclerotia is unlikely to infect plants located more than 2 cm from the parent sclerotium (Newton and Sequeira, 1972a). Since carpogenic germination of sclerotia and ascosporic infection are of greater importance with respect to rapeseed crops, myceliogenic germination and infection will be discussed whenever it is important.

Only preconditioned and functionally mature sclerotia are capable of producing apothecia (Abawi and Grogan, 1979; Purdy, 1979). The optimum conditions for preconditioning sclerotia have not yet been precisely determined, but it is generally recognized that newly formed sclerotia require holding for various lengths of time under cool, moist conditions before they are capable of maximum carpogenic germination (Abawi and Grogan, 1979). This time period is known to vary for different isolates of *S. minor* (Adams and Tate, 1976), so it is likely true for different isolates of *S. sclerotiorum* as well. The recorded time periods taken by sclerotia of *S. sclerotiorum* to produce apothecial initials vary from 13 to 208 days (Letham, 1975), indicating that there is no definite time period required. Observations in the field have also provided evidence that apothecial production is under environmental control, as sclerotia form apothecia at varying rates in different stands of rapeseed in the same year (Kruger, 1974, 1975a). Thus, the time period required for the preconditioning of sclerotia is likely more dependent on the interactions of several environmental variables than on any single factor or on any physiological differences between isolates. This ensures that apothecia and ascospores will only be produced when favourable for the ascospores to germinate and infect susceptible hosts (Willetts and Wong, 1980).

It must be remembered that carpogenic germination of sclerotia and apothecium formation are two different processes. Carpogenic germination refers to the initiation of stipes or carpophores on the sclerotium, whereas apothecium formation refers to the differentiation and expansion of the tip of the carpophores to form a disc-shaped ascocarp in which asci and ascospores will eventually be produced. The processes through which sclerotia germinate are (1) carpogenic germination and (2) myceliogenic germination.

8.10.1 Carpogenic Germination

If sclerotia are placed on a substrate low in nutrients (moistened sand, cotton or polyurethane, water agar or water) they will, under proper conditions, produce one or more stipes each with an apothecium. Low osmotic potential inhibits the formation of apothecia (Grogan and Abawi, 1975; Morrall, 1977). While some workers have recorded carpogenic germination in sclerotia taken directly from host tissue or laboratory media (Kosasih and Willetts, 1975), others conditioned sclerotia for several weeks or longer in a moistened state (Saito, 1973; Steadman and Nickerson, 1975).

The sequence of events for apothecium production from sclerotia has been studied by light and electron microscopy in conjunction with various histochemical tests (Jones, 1974a; Kosasih and Willetts, 1975; Saito, 1973). Apothecial initials arise in the cortex or medulla as brown to hyaline clusters or nests of interwoven hyphae. Active division of hyphae produces a knot of closely interwoven hyphae with dense cytoplasm. Eventually a few of the primordia erupt through the surface of the sclerotium. Next, clusters of microconidia are observed but there is no evidence that spermatization is a prerequisite for apothecial initiation (Kosasih and Willetts, 1975). Saito (1973) believed that, many primordia form but only a few erupt through the surface and the reserve materials of sclerotia can deplete if all the primordia develop. This suggests that some central mechanism(s) within sclerotia regulate the development of initials. Once the pointed, buff-coloured initials erupt through the surface, it continues to grow and develop. Stipe is positively phototropic and will not differentiate into disc unless receive light. Light below 390 nm is effective in inducing apothecium formation (Honda and Yanoki, 1977). A depression develops at the tip of the apothecial initials and paraphyses develop. Ascogenous hyphae with crosiers form in the subhymenium. After differentiation, the ascus contains eight ascospores (Kosasih and Willetts, 1975). The fully differentiated stipe consists of an outer layer of compressed hyphae and a medulla of elongated, septate, sparingly-branched hyphae. Following differentiation, the disc of the mature apothecium consists of (i) An ectal excipulum, (ii) A medullary excipulum, (iii) The hymenium, and (iv) The subhymenium (Kosasih and Willetts, 1975).

During carpogenic germination, the reserve materials are metabolized to provide "building blocks" which in turn are used for synthesis of the components of the stipe and apothecium. Intense activity of hydrolytic and synthetic enzymes in the area of the apothecial initials (Kosasih and Willetts, 1975) and β-glucanase enzyme have been recorded during germination (Saito, 1974a).

8.10.2 Myceliogenic Germination

Sclerotia may germinate by producing mycelia (Chet and Henis, 1975). *Sclerotinia* spp. has been shown to produce mycelia by hyphal or mycelial germination (Adams

and Tate, 1976). In hyphal germination, sclerotia placed on a substrate low in nutrients usually will produce sparse mycelial growth from hyphae which emerge though the rind. In some cases, this mycelial growth may produce smaller secondary sclerotia (Steadman and Nickerson, 1975). If hyphal germination occurs on a medium containing nutrients, the mycelia will continue to grow and produce new sclerotia. Mycelial germination has been observed with isolates that produce small sclerotia, e.g., *S. minor* (Adams and Tate, 1976). After a dormant period, sclerotia placed on a moistened medium develop one or more bulges. These bulges grow larger, finally rupture and release a plug or mass of dense mycelium that is visible to the unaided eye. In some instances two or more of these masses from the same sclerotia coalesce. Such mycelial masses grow from the sclerotia for distances of 2–3 mm.

8.10.2.1 Factors Affecting Myceliogenic Germination of Sclerotia

Myceliogenic germination is observed under 20–25°C, above 80 per cent relative humidity and initial medium pH of 5. In the absence of exogenous nutrients, sclerotia germinate more readily at 100 per cent RH than at 95 per cent RH or lower (Huang et al., 1998). Treatment with the root exudates of the sunflower hybrids significantly increases myceliogenic germination except during the flowering stage. Treatment with the amino acids alone has no significant effects on the myceliogenic germination, whereas in combination with peptone, significantly increases myceliogenic germination. Treatment with fungicides decreases myceliogenic germination, with benomyl treatment resulting in the highest reduction of sclerotia germination (88 per cent). Among the biological control agents, *T. viride* is the most effective biological control agent followed by *Bacillus* sp. Sclerotia produced at 15°C and stored for eight weeks at 4°C have the highest carpogenic germination (27.5 per cent) after incubation for four to six weeks in sand or vermiculite (Mosa et al., 2000a). Desiccation of sclerotia affects myceliogenic germination and hyphal growth. At 100 per cent RH or moist sand, desiccant-dried sclerotia germinate readily and produce vigorous hyphal growth, often develop into colonies (Huang et al., 1998).

8.11 Regulation of Stipe Production from Sclerotia

It has been suggested that many pigmented primordia remain dormant under the influence of some stipes initiated earlier in a sclerotium. Kapoor (1994) and Saito (1977) investigated the effect of removal of stipes on stipe production by sclerotia. It has been observed that repeated removal of stipes from germinating sclerotia result in the increased output of stipes in comparison with that produced on the control sclerotia. If stipes are removed, there is a four-fold increase in the total number of stipes produced over that observed when stipes are never removed (Fig. 8.11.1).

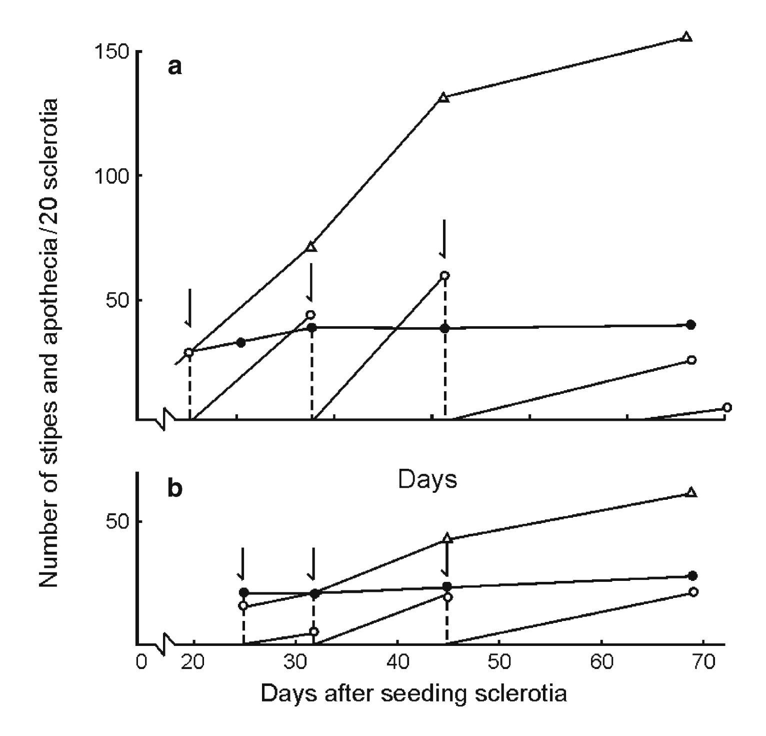


Fig. 8.11.1 Repeated stipe recovery from sclerotia after the periodic removal of stipes. (○) Number of stipes removed at each time (arrows); (▲) total number of stipes removed; (●) number of stipes and apothecia on the control sclerotia (Adapted from the publication of Saito, 1977. With permission)

This suggests an inhibitive growth correlation between stipes and the pigmented primordia in sclerotia. A similar inhibitive correlation has already been demonstrated by Henderson (1962a) between a stipe and its lateral branches.

The effects of numerous *in-vitro* and *in-vivo* factors will be considered as follows on the sclerotium germination and apothecium formation.

8.11.1 Effect of Nutrition

The germinability of sclerotia is found to be significantly affected by the kind of culture media upon which sclerotia are formed, even though there is no visible difference in the appearance, such as colour and shape. The most favourable medium for sclerotia to acquire a high germinability is bean leaf decoction

dextrose broth, followed by potato dextrose broth (Fig. 8.11.1.1). In contrast sclerotia produced on Houston's solution, a synthetic medium, germinate poorly, and though appear to be normally mature. It suggests that such conventional indicators of maturity as development of dark pigments or disappearance of liquid droplets over the surface of sclerotia do not necessarily indicate the ability to germinate. Thus, in the case of *Sclerotinia*, when a sclerotium can germinate to form apothecia, it is matured. The term "functional maturity" may be used to indicate such a situation. The most favourable nitrogen sources for functional maturation are found to be amino acids (Table 8.11.1.1). In contrast nitrate and ammonium nitrogen are not favourable, although the sclerotia produced are normally mature, at

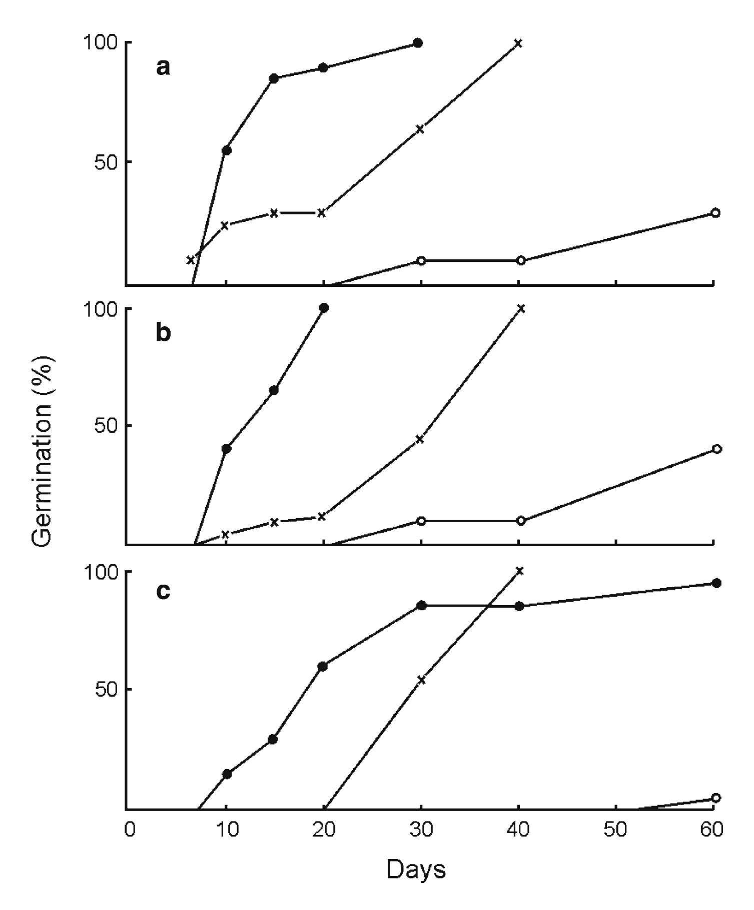


Fig. 8.11.1.1 Germination rates of sclerotia produced on storage media soaked with different nutrient solutions. Fresh weight of sclerotia (A) above 150 mg; (B) 150–100 mg; (C) below 150 mg (Adapted from the publication of Saito, 1977. With permission)

least from external appearance (Table 8.11.1.2). The C:N ratio also seems to affect "functional maturation" of sclerotia. The effects of carbon sources on sclerotial maturation have been examined using glutamic acid as the sole nitrogen source (Tables 8.11.1.3–8.11.1.7). Whenever a given carbon source is utilizable for mycelial growth and sclerotial production, sclerotia are functionally mature. Thus, sclerotia are produced on the medium containing all poly-, di- and monosaccharides except for sorbase on which the fungus does not grow and germinate normally. Polyols are not favourable for production of functional mature sclerotia except for sorbitol on which functionally mature sclerotia are produced. There is no relationship between the time and the "functional maturity" of sclerotia, i.e., percentage of maximum germination (Figs. 8.11.1.2, 8.11.1.3). It is suggested that under natural conditions "functional maturity" is altered in proportion to the difference of host plants or the growth stage of a single plant. The nature of "functional maturity" is obscure, but it is likely that this phase is different than that of

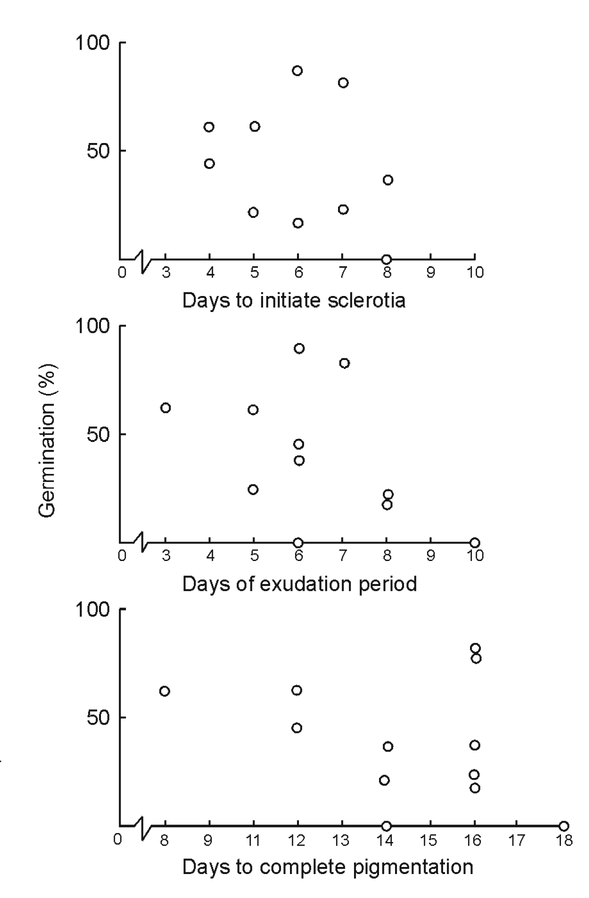


Fig. 8.11.1.2 Relation between the acquirement of germinability of sclerotia and the time to initiation and externally visible maturation of sclerotia produced on nitrogen nutritions (Adapted from the publication of Saito, 1977. With permission)

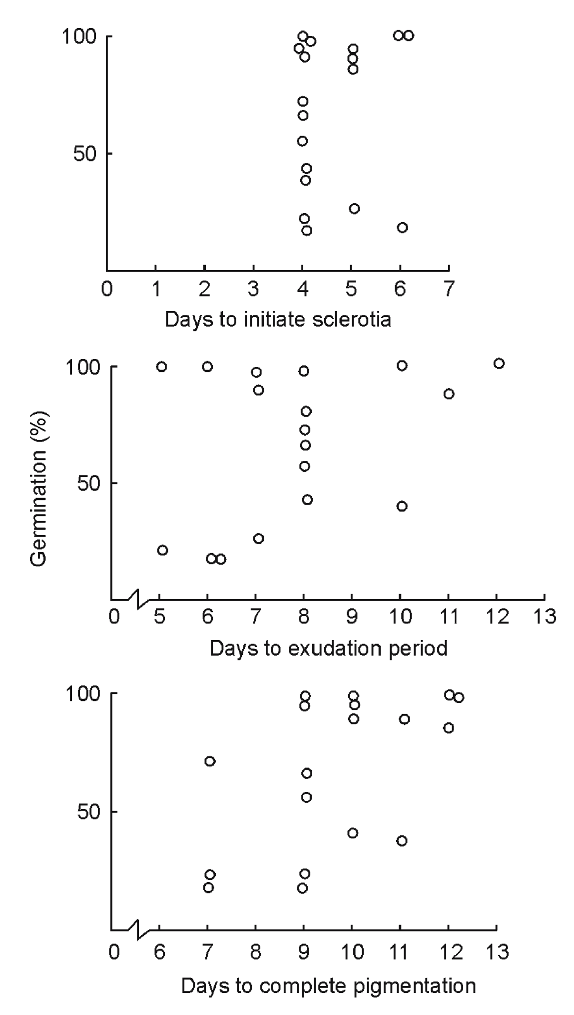


Fig. 8.11.1.3 Relation between the acquirement of germinability of sclerotia and the time to initiation and externally visible maturation of sclerotia produced on carbon nutritions (Adapted from the publication of Saito, 1977. With permission)

vegetative growth, since the addition of vitamins to a medium is usually favourable for mycelial growth (Tables 8.11.1.8–8.11.1.13) but not for "functional maturation" of sclerotia produced (Saito, 1977).

8.11.2 Effect of the Low Temperature Pretreatment

Sclerotia harvested from bean stem cultures were stored at 4°C, −10°C and −20°C with or without a water supply for one month, respectively. The sclerotia were placed at 15°C and their germination was compared to control sclerotia stored at

Table 8.11.1.1 Effects of various nitrogen sources on the production of sclerotia – amino acids (Adapted from the publication of Saito, 1977. With permission)

Nitrogen sources	Average number of sclerotia/Petri dish	Average dry weight of sclerotium (mg)
L-Alanine	22.4	7.0
γ-Aminobutyric acid	17.8	9.5
ε-Aminocaproic acid	0	0
L-Aspartic acid Na	19.8	6.8
L-Arginine HCl	14.1	5.7
L-Citruline	17.7	5.5
L-Cysteine	5.6	7.9
L-Cystine	2.7	4.6
L-Glutamic acid Na	11.7	17.2
Glycine	18.4	5.2
L-Histidine HCl	10.7	9.6
L-Homoserine	9.2	6.1
L-Leucine	10.8	6.6
L-Lysine HCl	0	0
L-Methionine	15.8	5.2
L-Ornitine	12.5	9.5
L-Proline	13.8	8.3
L-Phenylalanine	8.9	9.3
L-Threonine	10.0	2.6
L-Tryptophane	21.3	3.3
L-Tyrosine	16.1	8.6
L-Valine	7.8	8.0
NH_4NO_3	19.8	6.2
Bean leaf decoction dextrose agar	6.4	10.0

Nitrogen content, 245 mg/1,000 ml

Table 8.11.1.2 Effects of various nitrogen sources on the production of sclerotia- Ammonium salts and nitrates (Adapted from the publication of Saito, 1977. With permission)

Nitrogen sources	Average number of sclerotia/Petri dish	Average dry weight of sclerotium (mg)
NH_4NO_3	11.6	9.0
$(NH_4)SO_4$	13.3	9.3
$(NH_4)HPO_3$	8.3	8.2
CH_3COONH_4	12.8	5.1
$(NH_4)C_2\ O_4H_2O$	14.6	4.3
$(NH_4)HC_6H_6O_7$	11.5	9.8
$(NH_4)_2C_4H_4O_6$	13.1	4.6
KNO_3	13.7	12.2
$Ca(NO_3)_2$	14.2	13.1
$NaNO_3$	14.8	9.4
Casamino acids	7.8	19.4

Nitrogen content, 245 mg/1,000 ml

Table 8.11.1.3 Difference in the germinability of sclerotia produced utilizing various nitrogen sources – amino acids (Adapted from the publication of Saito, 1977. With permission)

	Germination (%) Days after seeding of sclerotia						
Nitrogen sources	14	22	27	34	40	47	56
L-Alanine	0	7.0	11.0	13.0	18.0	37.0	42.0
γ-Aminobutyric acid	0	2.0	11.0	14.0	38.0	71.0	72.0
L-Aspartica acid Na	0	0	0	1.0	2.0	65.0	76.0
L-Arginine HCl	0	0	0	0	0	2.0	3.0
L-Citruline	0	0	0	1.0	2.0	3.0	9.0
L-Cysteine	0	0	0	0	0	0	1.0
L-Cystine	0	0	0	0	0	0	0
L-Glutamic acid Na	0	4.0	6.0	15.0	47.0	67.0	70.0
Glycine	0	7.0	7.0	15.0	21.0	60.0	68.0
L-Histidine HCl	0	9.0	11.0	13.0	19.0	25.0	30.0
L-Homoserine	0	0	1.0	2.0	6.0	11.0	16.0
L-Methionine	0	2.0	3.0	10.0	16.0	44.0	54.0
L-Ornitine	0	0	0	0	1.0	1.0	1.0
L-Threonine	0	0	0	3.0	5.0	12.0	20.0
L-Tryptophane	0	1.0	5.0	8.0	11.0	20.0	24.0
L-Tyrosine	0	3.0	3.0	5.0	9.0	29.0	43.0
L-Valine	0	2.0	4.0	5.0	8.0	15.0	21.0
NH_4NO_3	2.0	8.0	14.0	16.0	21.0	44.0	53.0
Bean leaf decoction dextrose agar	5.0	10.0	16.0	25.0	45.0	75.0	100

Table 8.11.1.4 Difference in the germinability of sclerotia produced utilizing various nitrogen sources – ammonium salts and nitrates (Adapted from the publication of Saito, 1977. With permission)

	Germination (%) Days after seeding of sclerotia						
Nitrogen sources	8	14	21	27	34	56	69
NH_4NO_3	0	0	2.0	8.0	18.0	35.0	35.0
$(NH_4)_2SO_4$	0	0	0	0	0	0	1.0
$(NH_4)_2HPO_3$	0	0	0	2.0	7.0	20.0	22.0
CH_3COONH_4	0	0	1.0	30	4.0	9.0	9.0
$(NH_4)C_2\ O_4H_2O$	0	0	0	0	0	0	2.0
$(NH_4)_2HC_6H_5O_7$	0	0	0	0	3.0	6.0	12.0
$(NH_4)_2C_4H_4O_6$	0	0	3.0	7.0	8.0	19.0	31.0
KNO_3	0	0	0	0	0	5.0	10.0
$Ca(NO_3)_2$	0	0	0	3.0	10.0	18.0	19.0
$NaNO_3$	0	0	0	0	0	0	0
Casamino acids	0	0	1.0	4.0	15.0	27.0	27.0
Bean leaf decoction dextrose agar	34.0	87.0	99.0	99.0	100	100	100

Table 8.11.1.5 Effect of amino acid nitrogen on initiation and externally visible maturation of sclerotia (Adapted from the publication of Saito, 1977. With permission)

Nitrogen sources	Days of initiate sclerotia	Duration of exudation period (days)	Days of pigmentation
L-Alanine	6	8	15
L-Aspartica acid Na	5	3	8
L-Arginine HCl	4	5	11
L-Cysteine	8	6	13
L-Cystine	8	10	17
L-Glutamic acid Na	4	6	11
Glycine	8	6	15
L-Histidine HCl	5	8	13
L-Serine	7	7	15
l-Tryptophane	7	5	15
L-Tyrosine	6	6	15

Nitrogen content, 245 mg/1,000 ml

Table 8.11.1.6 Difference in the germinability of sclerotia produced on agar plates utilizing various nitrogen sources (Adapted from the publication of Saito, 1977. With permission)

	Germination (%)					
	Days after seeding of sclerotia					
Nitrogen sources	6	8	11	13	15	23
L-Alanine	0	0	0	1.5	1.5	18.5
L-Aspartic acid	10.1	26.1	44.2	52.2	63.3	63.3
L-Arginine HCl	30.3	54.9	57.4	57.4	60.7	63.0
L-Cysteine	0	0	0	0	0	0
L-Cystine	0	0	0	0	0	0
L-Glutamic acid Na	5.7	20.6	21.6	34.3	40.2	47.1
Glycine	4.0	19.2	27.3	32.3	34.3	38.4
L-Histidine HCl	2.9	5.8	7.9	9.4	14.4	22.9
L-Serine	34.3	51.8	77.4	78.8	82.5	82.5
L-Tryptophane	6.6	15.6	18.0	20.5	24.6	25.4
L-Tyrosine	77.6	83.2	87.9	88.6	88.6	88.6

Table 8.11.1.7 Effects of various carbon sources on the initiation, the number, the dry weight and the externally visible maturation of sclerotia – monosaccharides (Adapted from the publication of Saito, 1977. With permission)

Carbon sources	Average number/ Petri dish	Average dry weight of sclerotium (mg)	Days of initiation period (days)	Duration of exudation	Days of pigmentation
Arabinose	9.3	8.2	6	6	9
Fructose	8.0	11.9	4	8	10
Galactose	8.5	11.7	5	11	12
Glucose	7.0	13.5	4	10	10
Mannose	5.6	14.6	4	12	12
Ramnose	20.4	3.2	4	8	7
Sorbose	–	–	–	–	–
Xylose	18.1	1.6	5	7	9

Table 8.11.1.8 Effects of various carbon sources on the initiation, the number, the dry weight and the externally visible maturation of sclerotia – di and polysaccharides (Adapted from the publication of Saito, 1977. With permission)

Carbon sources	Average number/Petri dish	Average dry weight of sclerotium (mg)	Days of initiation	Duration of exudation period (days)	Days of pigmentation
Lactose	5.7	13.0	4	10	11
Maltose	12.9	7.7	4	8	10
Sucrose	5.4	17.0	6	5	12
Dextrin	11.4	8.7	4	8	10
Inulin	13.2	6.0	4	8	9
Soluble starch	5.1	11.4	5	7	11

Table 8.11.1.9 Effects of various carbon sources on the initiation, the number, the dry weight and the externally visible maturation of sclerotia – polyols (Adapted from the publication of Saito, 1977. With permission)

Carbon sources	Average number/Petri dish	Average dry weight of sclerotium (mg)	Days of initiation	Duration of exudation period (days)	Days of pigmentation
Galacitol	2.7	4.9	5	7	9
Erythritol	–	–	–	–	–
Glycerol	6.0	13.1	4	5	7
Inositol	1.8	1.7	6	6	9
Mannitol	1.7	2.1	4	6	7
Sorbitol	7.6	9.1	4	8	

Table 8.11.1.10 Difference in the germinability of sclerotia produced on agar plates utilizing various carbon sources (Adapted from the publication of Saito, 1977. With permission)

	Germination (%)					
	Days after seeding of sclerotia					
Carbon sources	6	8	10	12	14	16
Arabinose	27.5	59.5	79.0	94.0	98,5	99.0
Fructose	33.6	49.3	65.7	75.0	84.3	89.3
Galactose	30.2	43.6	49.7	57.7	73.8	85.9
Glucose	38.3	71.3	85.1	93.6	95.7	97.9
Mannose	39.1	60.0	79.1	82.6	88.7	97.4
Ramnose	6.9	20.6	29.9	39.5	54.8	71.0
Xylose	18.8	34.8	46.5	61.5	79.4	96.0
Lactose	7.1	21.4	24.1	33.9	34.8	38.4
Maltose	1.8	10.1	15.1	26.0	33.8	41.6
Sucrose	18.6	34.9	66.3	90.7	96.5	97.7
Dextrin	54.4	59.7	75.2	86.9	92.7	97.1
Inulin	4.9	19.2	23.6	38.9	40.9	55.2
Soluble starch	21.5	45.6	64.6	72.2	84.3	88.6
Galactitol	1.2	–	4.9	4.9	–	25.6
Glycerol	0	0	0	0	–	7.5
Inositol	0	0	0	1.2	0	16.7
Mannitol	0	0	0	0	–	16.1
Sorbitol	4.9	11.8	32.4	43.1	54.9	58.8

Table 8.11.1.11 Effect of vitamins on the production of sclerotia (Adapted from the publication of Saito, 1977. With permission)

Vitamins	Concentration (ppm)	Average number of sclerotia/Petri dish	Average dry weight of sclerotia (mg)
Biotin	0.05	10.9	11.2
Pyridoxine	0.1	13.9	12.2
Thiamine	0.1	10.9	7.4
Control	–	12.0	9.2

Table 8.11.1.12 Germination of sclerotia produced on the vitamin-added basal medium (Adapted from the publication of Saito, 1977. With permission)

	Germination (%)						
	Days after seeding of sclerotia						
Vitamins	8	14	21	27	34	56	69
Biotin	0	0	0	1.0	3.0	6.0	8.0
Pyridoxine	0	0	9.0	23.0	29.0	41.0	44.0
Thiamine	0	0	0	0	0	1.0	2.0
Control	0	0	2.0	7.0	20.0	35.0	38.0

Table 8.11.1.13 Effect of vitamins on the mycelial growth (Adapted from the publication of Saito, 1977. With permission)

Vitamins	Mycelial dry weight (mg)					
	Days of culture					
	3	6	9	12	16	22
Biotin	23.5	259.0	289.1	312.7	358.5	338.9
Pyridoxine	25.2	306.6	349.4	367.2	343.8	348.8
Thiamine	18.0	236.2	343.7	360.9	352.0	352.5
Control	36.0	49.9	34.6	104.4	227.9	246.8
Bean leaf decoction dextrose broth	36.0	234.9	219.0	223.3	215.6	215.8

room temperature (Fig. 8.11.2.1). The stimulatory effect of low temperature (chilling) at 4°C is evident when sclerotia are soaked in water and stored in a moist chamber through the pretreatment period. In contrast, if lacking moisture, there is no effect due to chilling and the germination rate at 15°C is almost the same as that of the control sclerotia. Pretreatment at −10°C and −20°C is found to be rather inhibitive to sclerotial germination at 15°C. The time required to reach maximum germination is apparently reduced (Fig. 8.11.2.2) with extension of the chilling period (Saito, 1977). Low temperature pretreatment of sclerotia is not essential for carpogenic germination (Coley-Smith and Cooke, 1971; Willetts and Wong, 1980). According to Huang (1991) when sclerotia of a Canadian isolate of *S. sclerotiorum* are exposed to −10°C or −20°C for four weeks their germination behaviour changes from carpogenic to myceliogenic type.

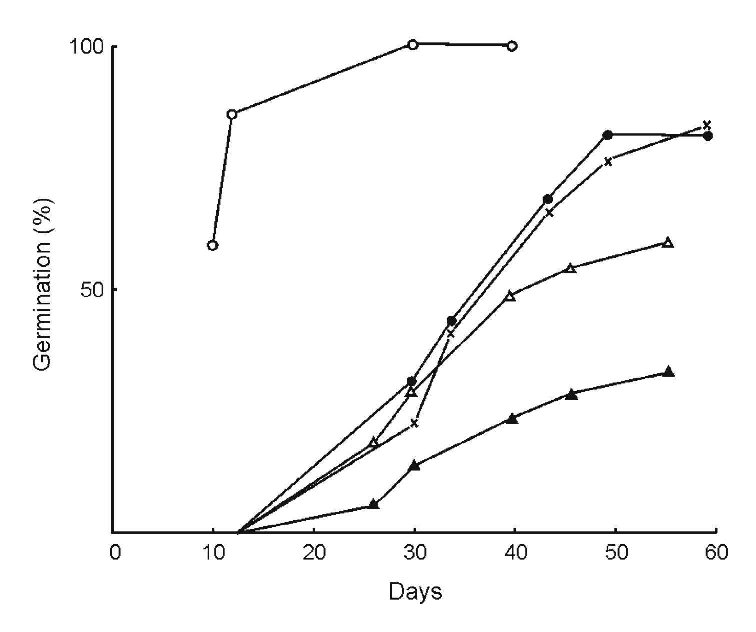


Fig. 8.11.2.1 Effect of pre-temperature treatments to sclerotia on the germination at 15°C. Temperature treatments: (○) 4°C moistened; (●) 4°C drying; (×) room temperature drying; (Δ) −10°C; (▲) −20°C (Adapted from the publication of Saito, 1977. With permission)

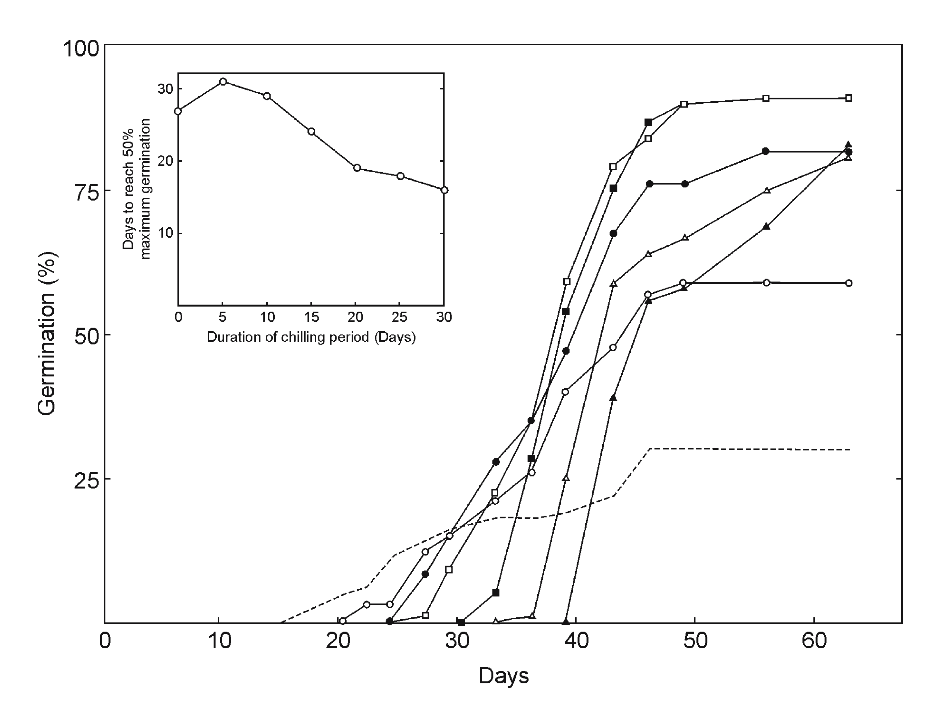


Fig. 8.11.2.2 Relation between the duration of low temperature treatments to sclerotia and the germination rate at 15°C. Duration: (○) 5 days; (●) 10 days; (□) 15 days; (■) 20 days; (▲) 30 days; (—-) control. Inset: Relation between the duration of chilling period and the velocity of sclerotial germination (Adapted from the publication of Saito, 1977. With permission)

8.11.3 Effect of Myceliogenic Germination

Although sclerotia of *Sclerotinia* spp. germinate to form apothecia, mycelial production is also frequently observed over the surface of sclerotia. This phenomenon is considered to occur as a renewed growth of inactive sclerotial cells and thus might be included in the category of germination. The germination of fungal sclerotia to form mycelia has already been termed “myceliogenic germination” (Coley-Smith and Cooke, 1971). This type of germination occurs in sclerotia seeded on moistened sponges or sand but more frequently when nutrients such as glucose or bean leaf powder are added to the medium, and apothecial production is inhibited (Table 8.11.3.1). There seems to be an antagonistic relation between the two modes of germination (Tables 8.11.3.2, 8.11.3.3). Thus, the production of apothecia is

Table 8.11.3.1 Inhibition of apothecial production (carpogenic germination) by mycelial growth from sclerotia (myceliogenic germination) (Adapted from the publication of Saito, 1977. With permission)

		No. of sclerotia germinated						
		Degree of mycelial growth						
Experiment	No. of sclerotia observed	0	1	2			3	
		A	M	A + M	M	A + M	M	A + M
1	48	32	2	5	2	1	2	1
2	66	31	3	5	2	3	12	0
3	54	34	0	5	4	3	8	0
Total	168	97	5	15	8	7	22	1

A = Number of carpogenic germination; M = Number of myceliogenic germination

Table 8.11.3.2 Percentage of myceliogenic and carpogenic germination of sclerotia in sterilized sand, sterilized and non-sterilized soil with organic amendments (Adapted from the publication of Saito, 1977. With permission)

		Days after seedling of sclerotia									
		6		10		14		27		42	
Media	Amendments	A[a]	M[a]	A	M	A	M	A	M	A	M
Sterilized sand	Non-glucose	0	11.0	2.0	27.0	9.0	30.0	15.0	29.0	41.0	34.0
	Bean leaf	0	26.0	0	46.0	1.0	46.0	1.0	82.0	–b	–
	powder	0	62.0	0	64.0	0	67.0	0	93.0	–	–
Sterilized soil	Non-glucose	0	10.0	5.0	21.0	7.0	21.0	–	–	–	–
	Bean leaf	0	13.0	0	19.0	0	35.0	–	–	–	–
	powder	0	28.0	2.0	36.0	2.0	[b]	–	–	–	–
Non-sterilized soil	Non-glucose	0	6.0	12.0	13.0	15.0	23.0	50.0	19.0	66.0	12.0
	Bean leaf	0	40.0	1.0	47.0	2.0	49.0	5.0	52.0	3.0	52.0
	powder	0	17.0	1.0	17.0	1.0	7.0	1.0	–[c]	1.0	–

[a] A = Carpogenic germination; M = Myceliogenic germination
[b] Further observation was interrupted by mycelial growth over the medium
[c] Mycelium of *S. sclerotiorum* was not distinguished from the another soil fungi over the soil

Table 8.11.3.3 Percentage of myceliogenic and carpogenic germination of sclerotia in non-sterilized soil with organic amendments (Adapted from the publication of Saito, 1977. With permission)

		Days after seeding of sclerotia							
		10		15		28		40	
Amendments		A	M	A	M	A	M	A	M
Glucose	1%	0	5.0	26.0	0	81.0	0	87.0	0
	2%	0	29.0	12.0	17.0	71.0	10.0	84.0	7.0
	5%	0	41.0	0	51.0	2.0	91.0	2.0	80.0
Bean leaf powder	1%	0	–	5.0	–	70.0	–	89.0	–
	2%	0	–	9.0	–	62.0	–	77.0	–
	5%	0	–	0	–	31.0	–	46.0	–
Rice bean	1%	0	–	23.0	–	75.0	–	79.0	–
	2%	0	–	5.0	–	77.0	–	89.0	–
	5%	0	–	4.0	–	54.0	–	70.0	–
Control		0	0	34.0	0	84.0	0	93.0	0

A = Carpogenic germination; M = Myceliogenic germination

suppressed in proportion to the degree of mycelial growth and in turn, little, if any mycelial growth is noted on sclerotia actively producing apothecia. The coexistence of the two modes of germination in a single sclerotium is rare. There is rapid exhaustion of reserves during "myceliogenic germination" of sclerotia in *Sclerotinia* spp. (Saito, 1977).

8.11.4 Effect of Soil Moisture

Moisture is an essential requirement for sclerotia to germinate and produce apothecia (Abawi and Grogan, 1975; Kruger, 1975a, b, 1976, 1980; Partyka and Mai, 1962; Williams and Stelfox, 1979). Free water is required, as stipes are not initiated even at 100 per cent RH (Coley-Smith and Cooke, 1971; Grogan and Abawi, 1975). A moisture content of 30 per cent in the soil is highly favourable for germination and apothecial formation (Singh and Singh, 1983). Sclerotia buried in heavy soils at 15°C germinate over a range of moisture levels from 15 to 50 per cent (Morrall, 1977). Sclerotia have been shown to germinate carpogenically over a range of water potentials from 0 to −7.5 bars (Morrall, 1977), which is in contrast to previous reports that germination is inhibited by even a slight moisture tension (Abawi and Grogan, 1975; Grogan and Abawi, 1975). The presence of ascospore infections on rapeseed crops in W. Canada (Duczek and Morrall, 1971; Morrall et al., 1976) proves that apothecia do develop in the field, so this is a clear indication that apothecia are formed at water potentials significantly less than 0 bars. Four techniques used by Teo and Morrall (1985a) showed that at matric potentials close to 0 bars, germination can occur only if sclerotia are on the soil surface and adequate oxygen is available. Buried sclerotia fail to germinate and many of them rot. The lowest matric potentials that appear to

induce carpogenic germination are –4, –7 and –15 bars using the inclined box, polyethylene glycol and sealed jar techniques respectively. Thus sclerotia can germinate in soil well below field capacity (Teo and Morrall, 1985b).

8.11.5 Effect of Temperature

Temperature affects both sclerotium germination and apothecium formation (Coley-Smith and Cooke, 1971; Kruger, 1976; Saito, 1977; Singh et al., 1985; Willetts and Wong, 1980). Mean values of temperature between 7°C to 11°C causes apothecia to develop best (Kruger, 1976). High temperature delays the germination, mycelial growth, initiation of sclerotial initials, exudation, pigmentation/maturation and reduces the number of sclerotia in culture plates (Singh et al., 1985). The amount and time of apothecial development is affected greatly by the degree of shading of the soil surface by the foliage of crops. It is the microclimate on the soil surface which is an important factor for apothecial development (Akai, 1981). Abundant stipe production occurs from sclerotia of *S. minor* incubated under a diurnal temperature regime of 15°C for 8 h and 10°C for 16 h, but no stipes are produced under diurnal regimes of 20°C/15°C and 25°C/20°C (Hawthorne, 1973). In later studies under controlled temperature conditions in the laboratory, Hawthorne (1976) found that, apothecial stipes are produced by sclerotia incubated at 10–19°C with an optimum at 15°C. Sclerotia germinate to produce stipes over a range of temperatures from 5°C to 25°C, but apothecial discs are formed only between l0°C and 20°C (Coley-Smith and Cooke, 1971; Saito, 1977; Willetts and Wong, 1980). The optimum temperature recorded for sclerotium germination is between 12°C and 18°C (Partyka and Mai, 1962) and 8–16°C (Dillard et al., 1995) respectively. With the increase in sclerotial size, there is increase in number of germinating sclerotia and apothecial sclerotium. Excessively cold or warm temperatures either inhibit germination or reduce the germination rate (Kruger, 1980). Soil temperatures lower than 10°C delays the appearance of apothecia in the field by reducing the frequency of sclerotium germination and by preventing the expansion of apothecial discs. Field experiments tend to support this claim (Kruger, 1973, 1975a, 1980) and overall spring temperatures have been positively correlated with the early or late appearance of apothecia in the field (Kruger, 1975a).

The effects of temperature and moisture are closely inter-related. Cool, moist conditions generally favour sclerotium germination and apothecium formation (Kruger, 1975a; Partyka and Mai, 1962; Walker, 1969), whereas high temperature combined with dry soils not only prevents sclerotium germination (Partyka and Mai, 1962), but also prevents any previously germinated sclerotia from forming apothecia since the stipes are unable to break through the hard surface layer of top soil (Kruger, 1976). Sclerotia exposed to extreme drying in the field suffer a reduced capability for carpogenic germination even after subsequently being exposed to optimum conditions, although they still remain viable (Abawi and Grogan, 1975; Grogan and Abawi, 1975). This may be because the cells responsible

for stipe initiation are not as resistant to desiccation as those responsible for myceliogenic germination (Abawi and Grogan, 1975). However, since both mycelia and carpophores are initiated from cells in the sclerotial medulla (Adams and Tate, 1976; Saito, 1977), it is not clear whether or not there actually are different groups of cells responsible for each mode of germination. Fifty per cent or more of the sclerotia in the soil are killed within 39 h at 40°C, 6 h at 45°C or 2 h at 50°C. When soils containing sclerotia are dried to a moisture of −1,516 bars or lower for seven days and remoistened to −0.2 bar, survival of the sclerotia and the viability at the soil surface and at depths of 0–2 cm declines during the summer months, whereas numbers of sclerotia at depths of 2–8 and 8–14 cm (Tables 8.11.5.1–8.11.5.3; Fig. 8.11.5.1) increases slightly and then remain constant (Adams, 1987b). However, according to Mitchell and Wheeler (1990) more apothecia are produced from sclerotia placed at 0–2 cm than from those buried deeper.

8.11.6 Effect of Light

Light is not required for sclerotia to germinate and produce stipes as these are produced in the dark as well as in the light (Bedi, 1962; Coley-Smith and Cooke, 1971; Letham, 1975; Natti, 1971; Purdy, 1956; Willetts and Wong, 1980). After emerging

Table 8.11.5.1 Time required to kill 50per cent of the propagules (LD_{50}) of three soil borne fungi in soil at various temperatures (Adapted from the publication of Adams, 1987b. With permission)

	LD_{50} (h)		
Temperature (°C)	*Sclerotium cepivorum* (sclerotia)	*Sporidesmium sclerotivorum* (macroconidia)	*Sclerotinia minor* (sclerotia)
35	129.6	266.4	624.0
40	9.5	37.2	38.4
45	1.7	5.2	3.4
50	0.8	0.8	1.5

Table 8.11.5.2 Survival of sclerotia (based on inoculum density) of *Sclerotinia minor* and *Sclerotium cepivorum* in moist soil (−0.2 bar) six weeks after infested (Adapted from the publication of Adams, 1987b. With permission)

	Survival based on percentage of control		
Matric potential of dried soils	*Sporidesmium sclerotivorum*	*Sclerotinia minor*	*Sclerotium cepivorum*
−0.2 bar (control)	100 a[a]	100 a	100 a
−1.160 bars	73b	77 ab	90 ab
−1.516 bars	61 c	69 bc	80 ab
−2.915 bars	39 d	30 c	59 bc
−3.656 bars	24 c	19 c	28 c

[a] Values in a column followed by the same letter are not significantly different according to Duncan's multiple range tests

Table 8.11.5.3 Survival of sclerotia of *Sclerotinia minor* in the field at various depths in the soil profile during the summer of 1985 (Adapted from the publication of Adams, 1987b. With permission)

	Percent survival of sclerotia at			
Sampling date	Soil surface	0–2 cm	2–8 cm	8–14 cm
8 May	100	100	100	100
20 May	83 cdef[a]	114 abcde	120 abcd	124 abcd
30 May	99 abcdef	137 abc	140 ab	136 abc
20 June	85 bcdefg	109 abcde	126 abcd	126 abcd
5 July	53 fg	113 abcde	131 abc	144 a
18 July	83 cdefg	115 abcde	140 ab	138 abc
30 July	36 g	94 abcdef	140 ab	140 ab
16 August	32 g	62 efg	126 abcd	140 ab
27 August	44 fg	72 defg	113 abcde	142 ab
15 September	32 g	53 fg	130 abc	125 abcd

[a] Values followed by the same letter are not significantly ($P \geq 0.01$) different according to Duncan's multiple range tests

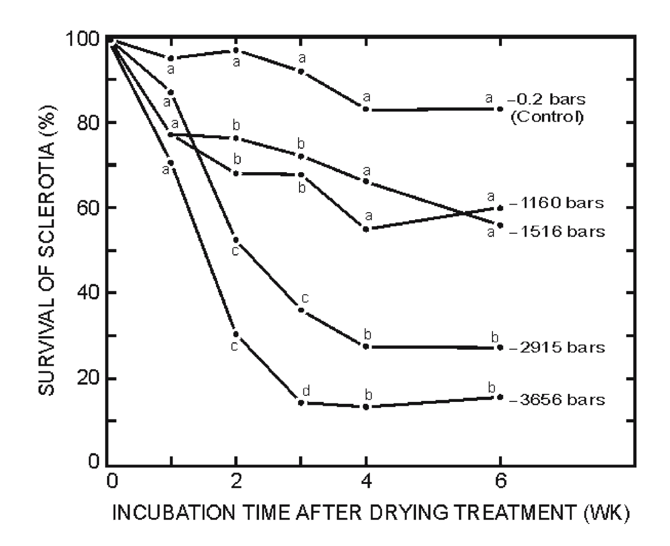


Fig. 8.11.5.1 Survival of sclerotia of *Sclerotinia minor* in soil after soil was dried to the indicated matric potential for seven days and remoistened to –0.2 bar for six weeks (Adapted from the publication of Adams, 1987b. With permission)

from the parent sclerotium, stipes are positively phototropic and bends towards the light (LeTournaeu, 1979; Willetts and Wong, 1980). The differentiation and full expansion of the apothecial disc only takes place in the light (Bedi, 1962; Coley-Smith and Cooke, 1971; Letham, 1975; LeTourneau, 1979; Natti, 1971; Purdy, 1956; Walker, 1969; Willetts and Wong, 1980) and at least 8–12 h of light out of every 24 h is required for this to occur (Bedi, 1962; Coley-Smith and Cooke, 1971).

Apothecial initials develop frequently at high light (120–130 mol m^{-2} s^{-1}) intensity (80 per cent) than at low light (80–90 mol m^{-2} s^{-1}) intensity. The apothecia are smaller at low light intensity than those produced at high light intensity (Sun and Yang, 2000). This dependence on light can be viewed as a mechanism by which the fungus insures that apothecial discs form above the soil surface, not below it, so that ascospores can be effectively dispersed (Willetts and Wong, 1980). As regards quality of light, white light is the best, green and red are very poor and blue is the worst (Bedi, 1962).

8.11.7 Effect of Sclerotium Size and the Depth of Sclerotium Burial in Soil

The results of several studies have shown that a positive correlation exists between sclerotium size and the number of apothecia produced (Bedi, 1963; Coley-Smith and Cooke, 1971; Kruger, 1974, 1975a). This is probably because large sclerotia have a correspondingly greater amount of stored nutrients in them that are used for apothecium formation. The depth at which sclerotia are buried in the soil also affects apothecial formation. The closer sclerotia are to the soil surface when they germinate, the more apothecia they produce (Akai, 1981; Kruger, 1975a; Singh and Singh, 1983; Willetts and Wong, 1980). Sclerotia are known to germinate at depths of 10 cm (Cooke et al., 1975; Willetts and Wong, 1980), but most sclerotia do not germinate unless they are nearer the soil surface (Kruger, 1980). If sclerotia are too deep when they germinate, the stipes cannot elongate enough to break through the surface layer of the soil and become exposed to the light required for the expansion of the apothecial discs (Willetts and Wong, 1980). Since apothecia with stipes longer than 3 cm are rarely produced under field conditions, it is likely that only sclerotia in the top 2–3 cm of soil are functional in producing apothecia (Abawi and Grogan, 1979).

8.11.8 Effect of Soil pH, Soil Textures, Soil Mixture and the Nutrient Status of the Soil

Sclerotia produced on media of differing pH's do not show any difference in their ability to produce apothecia (Bedi, 1963). However, the pH of the medium on which the sclerotia rest during germination affects germination and apothecium formation. The optimal range of pH for apothecium production is from 6 to 9.7 (Bedi, 1963; Coley-Smith and Cooke, 1971). Outside this range, sclerotia only produce stipes or do not germinate at all (Bedi, 1963). However, Hau et al. (1982) found a soil pH of 6.0 and 6.5 as the most conducive for sclerotial germination.

The best germination and mature apothecial formation is noticed in pure sand as compared to sandy-loam, silt-loam and also in different proportions of sand-clay mixtures. A sand-clay mixture of 3:1 gives the best sclerotial germination and apoth-

ecial formation. With the increase in clay proportion in such mixtures, sclerotial production and germination reduces (Singh and Singh, 1983; Mitchell and Wheeler, 1990). However, according to Kruger (1976) in marsh soils, apothecial development is higher than in sandy loam and loam from eastern Schleswig-Holstein. Size and burial of sclerotia of *S. sclerotiorum* directly affect the time taken for germination, stipe length, number of apothecia and apothecia diameter. Sclerotia germinate in 38 days when buried at 2 cm in clay loam soil and 62 days in river sand kept at 5 cm soil depth with larger (5.6 mm) apothecia (Singh and Tripathi, 1996c).

The nutrient status of the soil influences apothecium production including both the sclerotia formation and sclerotial germination. Sclerotia that form under nutrient rich conditions are sterile or very poor with respect to apothecium production. Those which form under nutrient-poor conditions only produce rudimentary or undersized apothecia. Optimal nutrient conditions for fungal growth results in formation of sclerotia that are very fertile with respect to apothecium production (Bedi, 1963; Willetts and Wong, 1980). Carpogenic germination of sclerotia is favoured by nutrient-poor conditions at the time of germination (Bedi, 1963; LeTourneau, 1979). If nutrients are present when sclerotia germinate, they tend to germinate myceliogenically, a process that is antagonistic to carpogenic germination (Bedi, 1963; Saito, 1977). Fewer sclerotia are produced in pots with added N and their production is delayed (Mitchell and Wheeler, 1990). The carpogenic germination of sclerotia is reduced in soils that contain high conc. of Na^+, Ca^{++} and SO_4^{2-} and low amounts of Mg^{++} and HCO_3 (Singh et al., 1995b).

8.11.9 Effect of Inhibitors

Differential inhibition has been recorded by Steadman and Nickerson (1975), i.e., different concentrations of an inhibitor often are needed to prevent stipe formation, apothecium formation and myceliogenic germination. A number of commercial fungicides and transition metal ions, especially cadmium are inhibitory in the range of 1 µM–1 mM. Higher concentrations (1–100 mM) of common buffers (acetate, phosphate and Tris), cations (sodium and lithium), anions (nitrate and sulphate) and low molecular weight carbohydrates (glucose, manitol, sucrose and trehalose), also inhibit germination. However, Singh et al. (1995b) reported that percentage of carpogenic germination of sclerotia of *S. sclerotiorum* is reduced in soils that contain high concentration of Na^+, Ca^{++}, Cl^- and SO_4^-. Similarly very low carpogenic germination has been observed in the soils that contain very low amounts of Mg^{++} and HCO_3^-.

8.11.10 Effect of Growth Regulators

To determine the effect of growth regulators, air dried sclerotia were soaked with and allowed to germinate in solutions of 1–100 ppm of gibberellic acid, kinetin,

Table 8.11.10.1 Effect of plant growth regulators on the germination of sclerotia (Adapted from the publication of Saito, 1977. With permission)

		% Germination[a]					
		Days after seedling of sclerotia					
Substances	Conc. (ppm)	17	19	21	23	27	31
β-(furyl) acrylic acid	1	0	1.3	8.0	14.7	20.0	26.7
	10	0	0	0	0	0	0
	100	0	0	0	0	1.3	2.7
Gibberellic acid	1	0	1.3	4.0	8.0	9.3	14.7
	10	1.3	1.3	2.7	8.0	10.7	13.3
	100	0	2.7	5.3	6.7	12.0	13.3
Indoll-3-acetic acid (IAA)	1	1.3	1.3	2.7	2.7	8.0	12.0
	10	0	0	0	0	0	0
	100	0	0	1.3	4.0	5.3	6.7
2,4-Dichloro-phenoxyacetic	1	0	0	0	0	5.3	10.7
acid (2,4D)	10	0	0	2.7	4.0	6.7	6.7
	100	0	2.0	2.0	2.0	2.0	6.0
Maleic hydrazide (MH)	1	0	0	4.0	5.3	12.0	14.0
	10	0	0	0	0	0	0
	100	0	0	0	0	1,3	4.0
2,4,5-Trichloro-phenoxyacetic	1	0	0	0	1.3	1.3	2.7
acid (2,4,5-TPA)	10	0	0	0	0	0	0
	100	0	0	0	0	0	0
Kinetin	1	0	0	0	1.3	8.0	8.0
	10	0	0	0	0	4.0	4.0
	100	0	0	0	0	0	0
Sclerin	1	1.3	10.7	12.0	21.3	29.3	37.3
	10	0	1.3	1.3	6.7	13.3	18.7
	100	22.7	34.7	44.0	54.7	60.0	66.7
Control	–	–	5.3	9.3	14.7	18.7	18.7

[a]Mean of three flasks

auxins, anti-auxins and sclerin. Of these, the only substance to stimulate germination (Table 8.11.10.1) is sclerin, a metabolite of *S. sclerotiorum* which is known to be a physiologically active substance (Saito, 1977).

8.11.11 Effect on Dry Weight

The dry weight of sclerotia decreases gradually but steadily with the production of apothecia (Saito, 1977). Sclerotan, a β-1, 3 glucan decreases markedly in parallel with apothecial production. The reduction in sclerotial dry weight is mainly due to the utilization of β-1, 3 glucans for apothecial production.

8.11.12 Effect of Enzyme Activity

Matured sclerotia have weak but detectable β-1, 3 glucanase and β-glucosidase activity, but there is no amylase or trehalase activity. With the beginning of germination, the activity of β-1, 3 glucanase apparently increases, whereas β-glucosidase activity remains at the initial level. The increase in β-1, 3 glucanase activity is most pronounced when apothecial stipes are actively produced. The activities of enzymes of carbohydrate metabolism have been compared between the different parts of germinating sclerotia where mature apothecia are seen, i.e., mature apothecia, stipes (immature apothecia) and sclerotia (Fig. 8.11.12.1). The specific activities of β-1, 3 glucanase and β glucosidase are highest in the sclerotia. At later periods of germination, when most stipes had developed into mature apothecia, detectable amylase and trehalase activity is found in the respective parts. In this state, the specific activity of amylase is highest in mature apothecia, but the trehalase activity is almost equal in the three parts. The highest activity of glucose-6-phosphate dehydrogenase is found in apothecia. By contrast, in germinating sclerotia, activity is very low, though somewhat higher than in ungerminating sclerotia (Fig. 8.11.12.2). These results clearly show the difference of physiological states between apothecia and sclerotia; the biosynthesis of materials needed for cellular construction is promoted in stipes and apothecia. In contrast, the catabolism of endogenous reserves takes place mainly in the medullary cells of sclerotia (Saito, 1977).

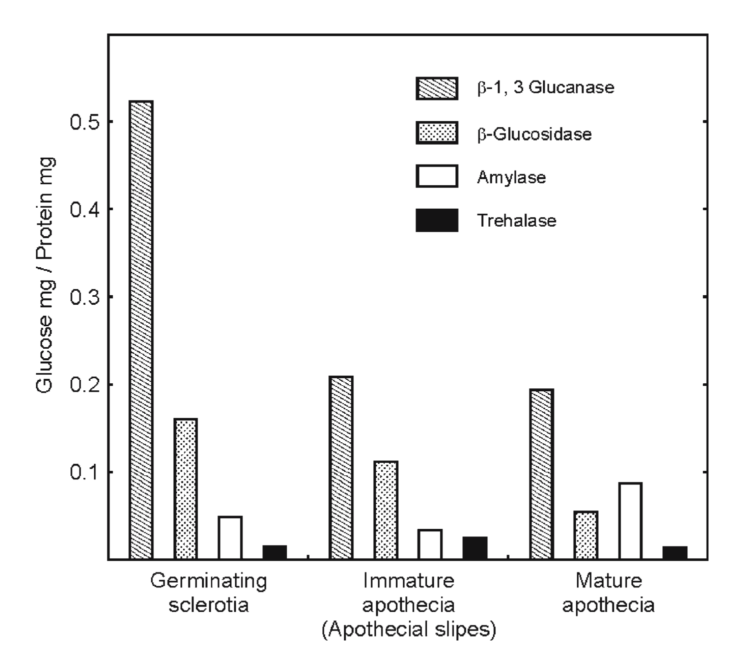


Fig. 8.11.12.1 Comparison between carbohydrase activities of germinating sclerotia, immature and mature apothecia (Adapted from the publication of Saito, 1977. With permission)

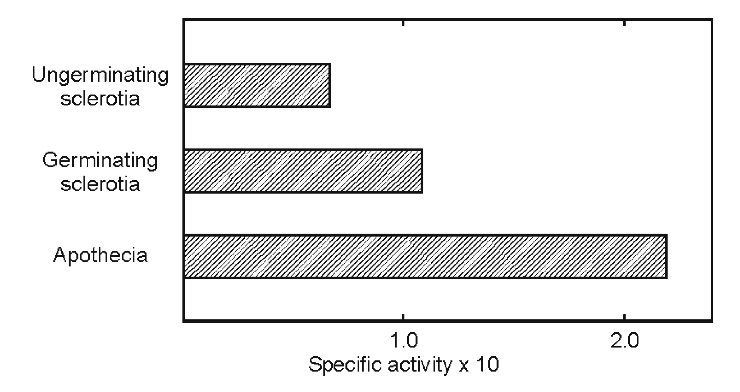


Fig. 8.11.12.2 Activities of glucose-6-phosphate dehydrogenase in ungerminating and germinating sclerotia and apothecia (Adapted from the publication of Saito, 1977. With permission)

8.11.13 Effect of Conditioning Medium and Period

Periods of conditioning in soil reduces the length of the resting period needed before sclerotia of *S. sclerotiorum* can germinate to form apothecia. Sclerotia from sunflower roots germinate sooner than those from the stem cavities. Germinability increases with the length of the conditioning period. Conditioning in soil is more effective than in moist vermiculite (Phillips, 1986b).

8.11.14 Effect of Host Exudates and Host Tissues

Root exudates from soybean cvs. significantly influence the number of apothecial formation (Chaves et al., 1996b). Germination of sclerotia of *S. minor* invariably increases in the presence of remoistened peanut leaves. Optimum stimulation is produced by 0.25–0.50 g of dried peanut leaves. Excessive peanut tissue (>1 g) tends to inhibit germination (Hau et al., 1982).

8.11.15 Effect of Cropping History

Previous cropping history significantly affects germination of sclerotia of *S. sclerotiorum*. Plots sown to rapeseed for two years contain more germinated sclerotia than plots sown to rapeseed for one year. Spring application of fertilizer increases germination early in the summer compared to with fall-application and no fertilizer treatment. Apothecial production generally follows the pattern for sclerotium germination and both indicate the potential for inoculum production of *S. sclerotiorum* ascospores under a non-host crop.

8.11.16 Effect of Crop Canopy

Light, soil moisture and soil temperature are all critical factors in determining sclerotium germination and apothecium formation and crop canopy influences all three of these factors (Williams and Stelfox, 1980a, b). Apothecium production is associated with the development of a canopy in bean fields (Schwartz and Steadman, 1978) and in rapeseed fields (Morrall and Dueck, 1982), thus providing evidence that the microclimate under the crop canopy is of major importance in the epidemiology of *S. sclerotiorum*. This is because the microclimate under a dense crop canopy remains relatively stable compared to the gross environment (Natti, 1971) and ambient temperature (Morrall and Dueck, 1982) combined with high water potentials in the upper layers of soil (Abawi and Grogan, 1979) certainly favour sclerotium germination and apothecium formation.

8.11.17 Effect of Other Micro-organisms

A number of antagonistic micro-organisms are present in the field. Their role in the survival and germination of sclerotia and formation of apothecia has been discussed in the biological control section (19.9, 19.10).

8.11.18 Effect of Fungicides and Herbicides

The fungicides Bavistin (50 ppm), Topsin M (100 ppm), Sailaxyl MZ (100 ppm), mancozeb (1,000 ppm) and neem extract (5,000 ppm) completely inhibits sclerotial growth placed on PDA (Zewain et al., 2004). However, carbendazim (100 µg/ml for 20 min) is the most effective in suppressing carpogenic germination of sclerotia (Singh et al., 2003). The fungicides Ridomil G, Benlate, Tecto 60 and Topsin M @ 100 ppm reduce mycelial growth (Iqbal et al., 2003). The application of fungicides and herbicides under field conditions affects sclerotial survival, germination and apothecial formation. The specific effects have been discussed in Sections 19.3, 19.6.

8.11.19 Influence of Different Irrigation Regimes on Carpogenic Germination of Sclerotia of Sclerotinia

Apothecia production starts after 25–30 days of continuous soil moisture and is strongly influenced by the irrigation regime. Apothecia development is delayed if an irrigation break occurs before the start of apothecia production. The delay is approximately of the same length as the irrigation break. A high irrigation level

caused more abundant apothecia production in the sand than in the loam, while at a low irrigation level, more apothecia are produced in the loam than in the sand. The maximum life span of apothecia is 33 days which is measured in the sand irrigated at 5 mm/day. The length of the moist period needed for carpogenic germination and the influence of dry periods can be used when predicting the start of apothecia development. It is also shown that both soil type, amount of irrigation or rainfall and its distribution must be considered when estimating the number of apothecia and apothecia longevity (Twengstrom et al., 1998b).

8.11.20 Effect of Age of Sclerotia

Immature sclerotia (light brown to grayish black with sacs of liquid on the surface, from 6- and 8-days old cultures) readily germinate myceliogenically, producing hyphae, but mature sclerotia (black and dry, from 14-, 21- and 42 days old cultures) germinate carpogenically, producing apothecia directly. Germination responses of mature sclerotia from 14- to 42-days old cultures of two isolates are similar (Huang and Kozub, 1994).

8.12 Ascospore Discharge and Dispersal

During the process of apothecium maturation, ascospores are forming within them. Immature apothecia contain few ascospores (Kruger, 1974, 1975a), but it has been estimated that a mature apothecium can form and discharge as many as 2.32×10^6 ascospores over a period of a week or more under favourable conditions (Schwartz and Steadman, 1978). Since a single sclerotium can produce up to 100 apothecia, a single sclerotium thus has the potential to produce as many as 2.3×10^8 ascospores (Schwartz and Steadman, 1978). When *S. sclerotiorum* apothecia i.e., “puff” develops fully, the rate of discharge of ascospores increases rapidly to a maximum which is maintained briefly and then declines gradually; individual asci continue to dehisce for several seconds after the main discharge. The discharge of *S. minor* apothecia is more ephemeral. The maximum discharge rate appears to reach as soon as the puff starts and the discharge ends abruptly (Hartill and Underhill, 1976).

Mature asci forcibly discharge ascospores into the air for a distance of 1 cm or more, thereby enabling the ascospores to escape the still boundary layer of air near the soil surface and to reach the more turbulent air above (Abawi and Grogan, 1979). Ascospores are released continuously in turbulent air, but in still air ascospores are released in bursts of about 0.1 s duration, forming elongated columns of ascospores in the air above the apothecium. Mass discharge occurs in still air because it is a more efficient mechanism by which ascospores can be ejected through the still air layer, insuring that at least some ascospores will be carried away by whatever slight air currents are present (Willetts and Wong, 1980).

Apothecia require at least 98 per cent RH in order to remain turgid and will collapse at lower RH's, although recovery can occur if the apothecia are returned to an environment of 100 per cent RH (Partyka and Mai, 1962). High temperatures and dry soils dehydrate apothecia and thereby prevent ascospore discharge, so dry apothecia are consequently of little importance as a source of inoculum (Kruger, 1974, 1975a, 1976, 1980; Williams and Stelfox, 1980b). As long as moisture is not limiting, temperature alone will influence ascospore discharge. Ascospore discharge is optimum at 22°C and occurs over a temperature range from 4°C to 32°C (Newton and Sequeira, 1972a). Lower than optimum temperatures greatly reduce the number of ascospores released and almost no ascospores are released at 4°C. Higher than optimum temperatures also inhibit ascospore discharge, but whereas apothecia can recover from prolonged low temperature after being returned to optimum conditions, high temperatures have a permanent detrimental effect (Newton and Sequeira, 1972a).

Field experiments have shown that many ascospores are discharged during warm, windy weather as long as the apothecia remain turgid and that few ascospores are discharged in humid and rainy weather (Kruger, 1975a). Rain prevents ascospores from being discharged into the air instead, the ascospores diffuse into the films of water present on the apothecia during rain showers and are eventually washed into the soil (Kruger, 1975a, 1980). Heavy rains can therefore, significantly reduce the level of disease in a rapeseed crop if they occur at a time coincident with ascospore discharge and situations where this has happened have been documented (Kruger, 1975a).

After being discharged from apothecia, ascospores can be carried in a viable condition for several kilometers by wind (Abawi and Grogan, 1979; Walker, 1969; Williams and Stelfox, 1979). Evidence for this comes from work done on beans (Akai, 1981; Abawi and Grogan, 1975) and on rapeseed (Williams and Stelfox, 1979, 1980a), where disease development is observed in fields where the only apothecia found are some distance outside the fields involved. Spore trapping studies have also shown that ascospores are present up to 150 cm above the soil surface (Williams and Stelfox, 1979), which is more evidence that ascospores can be carried for some distance by wind. Thus, wind blown ascospores are a major means by which field to field spread of disease may occur. Ascospores are also present on pollen grains, some of which are carried by bees (Stelfox et al., 1978), but this is of minor importance in the spread of disease (Williams and Stelfox, 1979).

Ascospore dispersal appears to be influenced by only two major factors. Firstly, at least some wind must be present to carry the ascospores to susceptible hosts. Secondly, the crop canopy itself may restrict ascospore dispersal by preventing the ascospores from entering air currents above the crop canopy. Different crops probably have different abilities to restrict ascospore movement (Williams and Stelfox, 1979).

8.13 Ascospores Survival

Ascospores survive the longest when they are dry, indicating that survival is likely determined by a close relationship between both temperature and moisture. Ascospores are known to survive as long as 12 days in the field (Grogan and Abawi, 1975).

However, ascospores remain viable for two to five months under greenhouse conditions. Ascospores remain viable for greater lengths of time at 60 per cent RH than at 80 and 98 per cent RH (Newton and Sequeira, 1972a; Partyka and Mai, 1962; Scheibert-Bohm et al., 1981). Temperature of 25°C or greater combined with RH in excess of 35 per cent are most detrimental to *S. sclerotiorum* ascospore survival. Generally, ascospore mortality increases as temperature and RH increases. After 24 h of irradiation with two FS-40 sunlamps in the laboratory, ascospore survival is 49 per cent and 13.4 per cent under plastic films that transmit 10 and 50–60 per cent UV radiation in the 300–400 nm range respectively (Caesar and Pearson, 1983).

8.14 Ascospore Germination

Temperature does not seem to have much effect on ascospore germination, most ascospores will germinate within a period of 6 h under laboratory conditions over a temperature range from 10°C to 30°C (Abawi and Grogan, 1975). The optimum temperature for germ tube growth and for initiation of infection is between 20°C and 25°C, with a reduction in growth rate occurring at higher and lower temperatures (Abawi and Grogan, 1975).

Water potentials as low as −56 bars also do not adversely affect either the rate of germination or the percentage of germination, but even lower water potentials reduce germination and a water potential of −91 bars completely inhibits it (Grogan and Abawi, 1975). However, free water is an absolute requirement for host infection to occur. A minimum of 48 to 72 h of continuous leaf wetness is required for ascospores to infect beans, and that RH values near 100 per cent are not sufficient for infection to occur (Abawi and Grogan, 1975). Free water at the ascospore-host tissue interface is required more for the growth of germ tubes than for the actual germination of the ascospores. However, laboratory work has shown that the growth of germ tubes does not require a water potential of 0 bars, but is actually stimulated by water potentials from −1 to −14 bars (Grogan and Abawi, 1975). However there is need to generate more information on this aspect.

8.15 Ascospore as Inoculum

Germinating ascospores have been reported to penetrate healthy tissues directly (Purdy, 1979). The process of ascospore germination and host penetration both require energy and although there are enough stored nutrients in ascospores for them to germinate and form germ tubes, but there are not enough energy reserves for the formation of appressoria (Lumsden, 1979). Thus an exogenous source of nutrients in the infection court is required before host penetration can occur (Abawi and Grogan, 1975, 1979; Purdy, 1958; Willetts and Wong, 1980) and dead flower parts have been shown to play a major role in this regard (Abawi and Grogan, 1979;

Abawi et al., 1975; Akai, 1981; Kruger, 1975b; McLean, 1958b; Natti, 1971; Purdy and Bardin, 1953). Pollen grains also provide a rich source of nutrients and stimulate ascospore germination (Stelfox et al., 1978). Wounds or senescent tissues still attached to the plant also provide a nutrient base for ascospore germination and subsequent host penetration (Abawi and Grogan, 1979; Newton and Sequeira, 1972; Willetts and Wong, 1980). Details of process of host infection have been covered in the infection and pathogenesis section as well as in epidemiology of different *Sclerotinia* diseases (12, 16).

8.16 Calcineurin for Sclerotial Development and Pathogenicity

Sclerotinia sclerotiorum is a necrotrophic, omnivorous plant pathogen with worldwide distribution. Sclerotia of *S. sclerotiorum* are pigmented, multi-hyphal structures that play a central role in the life and infection cycles of this pathogen. Calcineurin, a Ser/Thr phosphatase linked to several signal-transduction pathways, plays a key role in the regulation of cation homeostasis, morphogenesis, cell-wall integrity and pathogenesis in fungi. Calcineurin expression in *S. sclerotiorum* is altered in a phase-specific manner during sclerotial development. Inhibition of calcineurin by FK506, cysclosporin A, or inducible antisense calcineurin expression impairs sclerotial development at the pre-maturation phase and increases germination of preformed sclerotia. Induction of antisense calcineurin expression in *S. sclerotiorum* results in reduced pathogenesis on tomato and *Arabidopsis*. However, secretion of oxalic acid, a key virulence factor of *S. sclerotiorum* is not altered. Inhibition of calcineurin conferred a reduction in cell wall β-1, 3-glucan content and increased sensitivity to cell wall degrading enzymes and to the glucan synthase inhibitor caspofungin. Thus, calcineurin plays a major role in both sclerotial development and pathogenesis of *S. sclerotiorum* and most likely, other phytopathogens (Harel et al., 2006).

8.17 Effects of Exudates Depletion on Sclerotial Development

Exudates depletion from *Sclerotinia sclerotiorum* sclerotia causes poor development of sclerotia, causing reduced sclerotial size and less compactness of the peripheral cell layers. Exudates also show antifungal activity against some parasitic as well as saprophytic fungi. *Aspergillus flavus*, *Cercospora blumea*, *Colletotrichum capsici* and *Fusarium udum* show maximum sensitivity to exudates in undiluted form where less than 40 per cent conidial germination is recorded. High performance liquid chromatographic (HPLC) analysis of the ethyl acetate fraction of exudate shows that it consisted of tannic, gallic, ferulic and cinnamic acids along with many other unidentified compounds. The exudate-depleted sclerotia are able to cause infection in egg plants (*Solanum melongena*) in glasshouse. Foliar application of oxalic acid and

sclerotial exudate of *S. sclerotiorum* induce the synthesis of phenolic acids (gallic and cinnamic acid) in treated eggplant leaves. Maximum induction of gallic acid is seen in leaves treated with 0.1 per cent oxalic acid after 96 h, whereas maximum induction of cinnamic acid is observed in leaves treated with undiluted exudates after 48 h. The lesser amount of cinnamic acid synthesis in leaves in presence of mycelia of *S. sclerotiorum* indicates the operation of some other resistant reactions other than the phenyl propanoid pathway (Singh et al., 2004).

8.18 Effect of Rind Damage and Regeneration on Permeability of Sclerotia

Sclerotia of *S. minor* have the ability to regenerate a new rind when the old rind is damaged before or after maturity. Regeneration involves growth of a completely new rind to cut off the exposed damaged surface. New, more or less spherical rind and cortical cells form from outgrowths of existing medullary hyphae. The new rind cells become pigmented over several days and new cortical cells contain reserves when the new rind has become fully pigmented. This process is most rapid in immature sclerotia but even in mature sclerotia, a new rind is fully regenerated within eight days. Intact mature sclerotia exclude the apoplastic tracer sulforhodamine G but when the rind is damaged they become leaky to the fluorochrome. Regeneration of a new rind reduces permeability to sulforhodamine but the initial impermeable state is not fully regained, even when regeneration is complete. Sclerotia that have been damaged in the field, despite regeneration, might be more susceptible to external influences than those in which the rind has remained intact (Young and Ashford, 1996).

Chapter 9
Ultrastructures

Electron microscopy in association with physiological, biochemical and genetical studies has provided information which helps in understanding the complex host-parasite relationship of the disease.

9.1 Sclerotial Maturation

Although there is an ontogenetic diversity among species, the development of fungal sclerotia can be divided into three phases: initiation from vegetative hyphae, increase in size and maturation (Townsend and Willetts, 1954; Butler, 1966). In *S. sclerotiorum*, mature sclerotia consist of a well differentiated outer rind and a medulla of prosenchymatous hyphae. Previous light microscopic study showed that primordia of apothecial stipes developed from the medullary hyphae beneath the rind of the germinating sclerotia (Saito, 1973). Thus, this type of germination of sclerotia which is designated as "carpogenic germination" (Coley-Smith and Cooke, 1971) is initiated by the cellular differentiation, presumably in a way quite different from the vegetative cell growth of certain medullary hyphae.

9.1.1 Tissue Differentiation of Sclerotia and Ultra-structural Changes of Component Cells

The marginal cells of immature sclerotia lose their filamentous nature by repeated septation (white sclerotia) and the outermost cells change into slightly thick-walled, globose cells (slightly pigmented sclerotia). This is followed by the differentiation of rind consisting of dark pigmented, thick-walled cells. At this time sclerotia are easily separated from the underlying colony (mature sclerotia). In parallel with rind differentiation, the inner cells retain their filamentous nature, but a layer which is not stained with dye gradually develops around them (Plate 9.1.1.1) and the large central zone of sclerotia is finally occupied with such cells to form a medullary tissue (Saito, 1974b, 1977).

G.S. Saharan, Naresh Mehta, *Sclerotinia Diseases of Crop Plants: Biology, Ecology and Disease Management*,

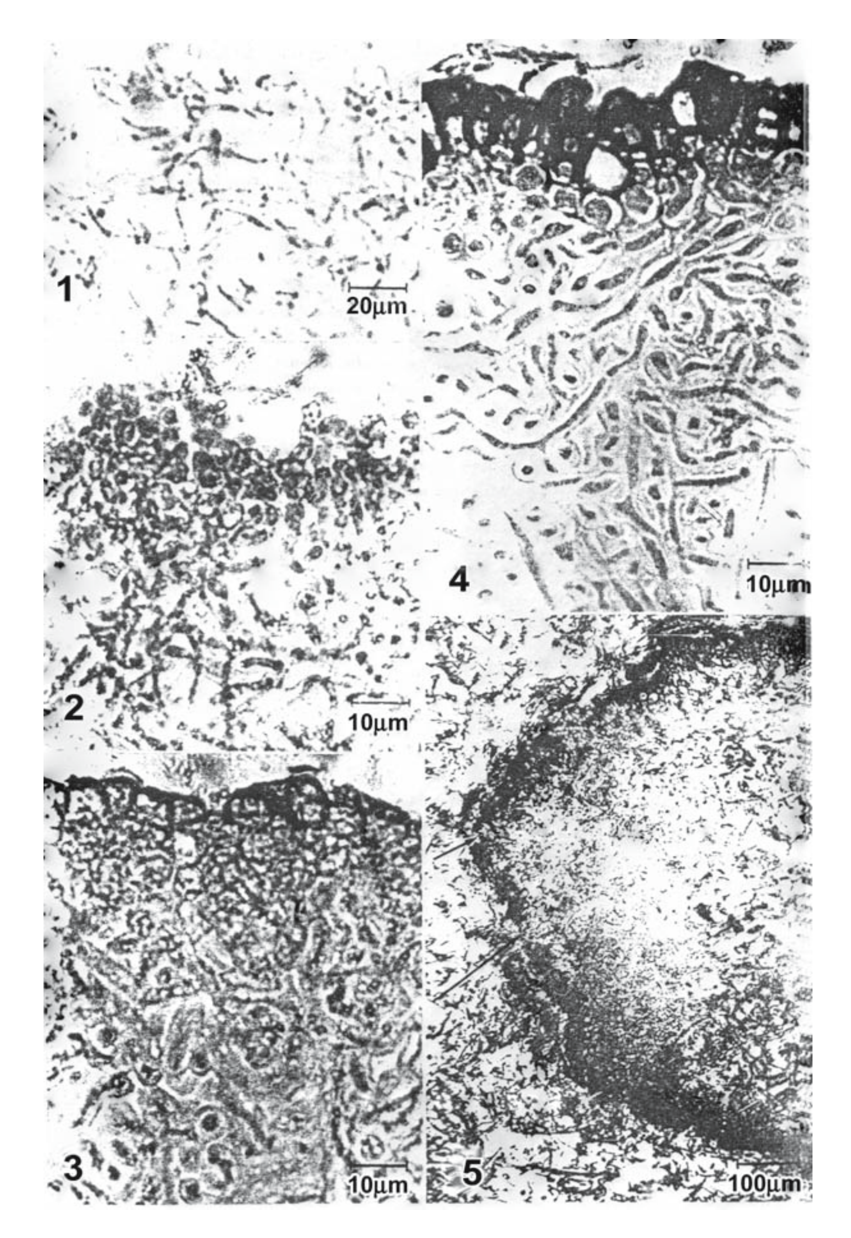


Plate 9.1.1.1 Light micrographs of vertical sections of sclerotia. (1) A sclerotium at earlier stage of development; (2) A white sclerotium; (3) A slightly pigmented sclerotium; (4) A mature sclerotium; (5) A sclerotium germinated to form mycelium. Rind is partially destroyed (arrows) (Adapted from the publication of Saito, 1977. With permission)

9.1.1.1 Changes in the Cell Wall

The cell wall of vegetative hyphae appears to have a one layered, homogenous zone from 0.09 to 0.18 μm in thickness. A simple single perforated septum characteristic of ascomycetous fungi is present in Plate 9.1.1.1.1. In white sclerotia, the cell wall

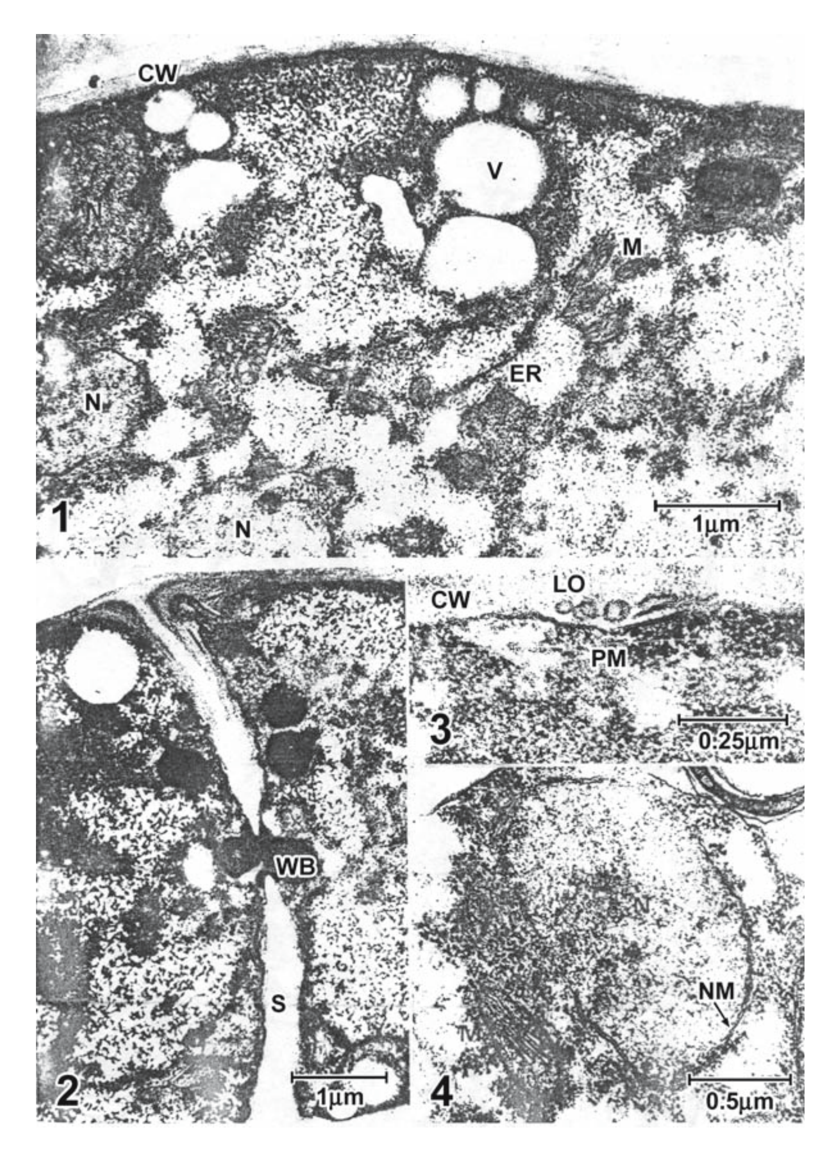


Plate 9.1.1.1.1 Ultra-thin sections of vegetative hyphae; (1) A part of hyphal cell; (2) A septum (S) associated with woronin bodies (WB); (3) Lomasome (LO) between the cell wall (CW) and plasma membrane (PM); (4) Nucleus (N) with double nuclear membrane (NM) and mitochondrion (M) (Adapted from the publication of Saito, 1977. With permission)

of most of the inner cells (medullary cells) has a thickness similar to that of vegetative hyphae. However, they are enveloped with a fibrous material in various degrees and in some cases the fibrous material develops into a conspicuous layer (Plate 9.1.1.1.2). Subsequently, with the development of the fibrous layer, thickening of the

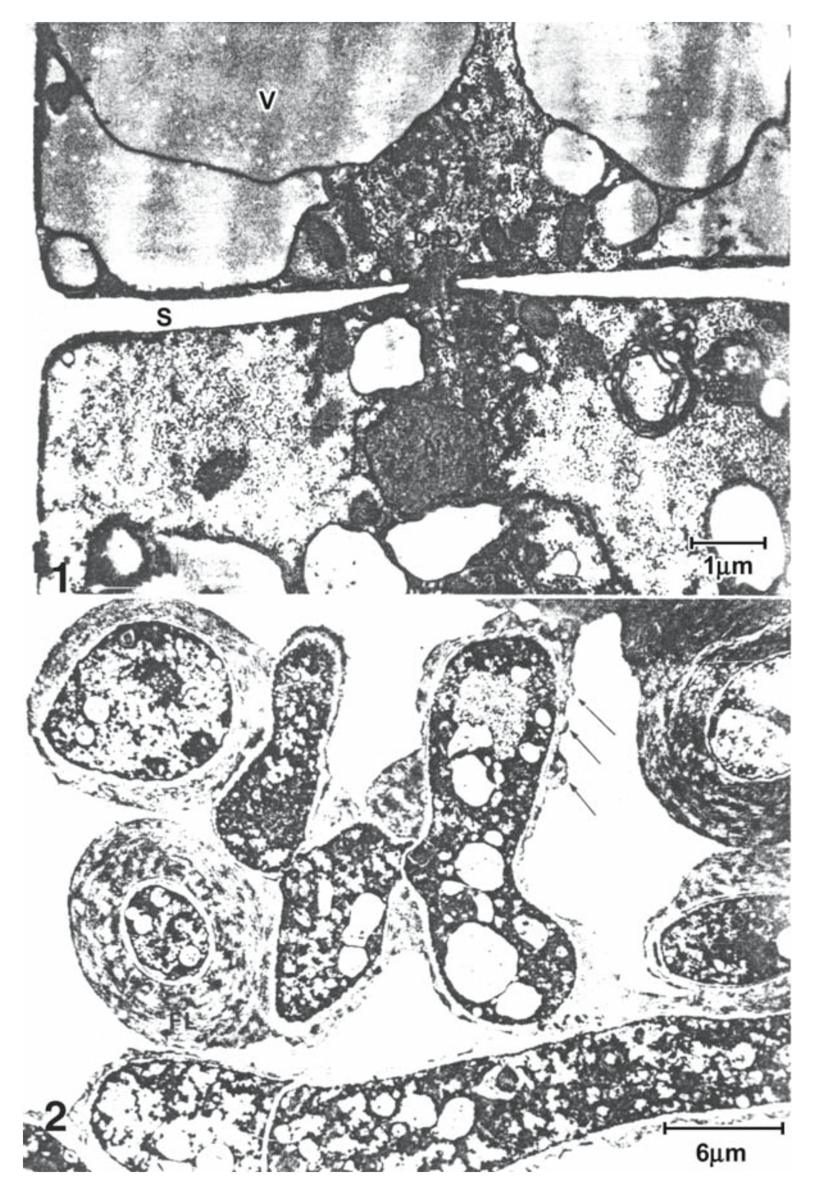


Plate 9.1.1.1.2 (1) Ultra-thin section of vegetative hyphae showing a septum (S) with simple pore and electron dense deposition (DED) on the pore rim. (2) A typical ultra-thin section of a white sclerotium. Note the outgrowth of fibrous layer (arrows) (Adapted from the publication of Saito, 1977. With permission)

cell wall progresses. In slightly pigmented sclerotia, the development of such a fibrous layer also terminate and in some cases its outer surface is further enveloped with a very thin, electron dense layer, even though cell wall thickening still appears to continue (Plates 9.1.1.1.3, 9.1.1.1.4). As the result of such changes, medullary cells of mature sclerotia have two major layers, a thickened homogenous cell wall and a much thickened, fibrous layer corresponding to the non-stainable layer which is observed under a light microscope (Plate 9.1.1.1.5). A non-cellular matrix is lacking and thus intercellular spaces are evident (Saito, 1974b, 1977; Calotelo, 1974).

9.1.1.2 Changes in Cytoplasm

Various organelles common in fungi are seen in the cytoplasm of vegetative hyphae, mitochondria, ribosomes, vacuoles, endoplasmic reticulum, lomasomes etc. (Plate 9.1.1.1.1). The septal pore is often seen associated with a small deposit of electron dense, amorphous materials on the pore rim and with a number of electron dense woronin bodies (Plates 9.1.1.1.1-Fig. 2, 9.1.1.1.2-Fig. 1). Cytoplasmic features of medullary cells of white sclerotia are generally similar to that of vegetative hyphae, except that electron dense materials disappear on the pore rim and lomasomes are seen to have more developed structure as multi-vesicular and multi-tubular bodies (Plate 9.1.1.2.1). Significant changes occur in mitochondria and vacuoles during sclerotial maturation. Mitochondria with distinct cristae, usually more elongated, are still abundant in the medullary cells of slightly pigmented sclerotia (Plate 9.1.1.1.3-Fig. 3). However, the cristae of mitochondria in mature sclerotia are indistinct, and thus it is not easy to discern this organelle at this stage (Plate 9.1.1.1.4-Fig. 2). Such a change in the mitochondrial structure reflects the decrease of oxygen uptake by sclerotia with maturation (Fig. 9.1.1.2.1). In vacuoles, electron dense, amorphous materials are deposited and their volume increases as sclerotia mature (Plates 9.1.1.1.3, 9.1.1.1.4). In mature sclerotia, numerous vacuoles almost filled with such materials are evident (Plates 9.1.1.1.4-Fig. 2, Plate 9.1.1.2.2) (Saito, 1977; Calotelo, 1974).

Bullock et al. (1980a) described a detailed light and electron microscopic investigation of the development and structure of sclerotia of *S. minor* having smaller sclerotia than *S. sclerotiorum* and *S. trifoliorum*. The sclerotia of *S. minor* are formed beneath a weft of overlying vegetative hyphae that sometimes become enveloped as the sclerotium enlarges. Differentiation of the sclerotial hyphae into regions of rind, cortex and medulla begin only 12–24 h after sclerotial initiation occurs. The cortex is the last region to become discernible. The rind consists of a closely packed layer of cells around the sclerotium. The cortex is about three cells wide and is made up of pseudoparenchymatous tissue. The prosenchymatous medulla constitutes the main part of the sclerotium. Cytoplasmic reserves tentatively identified as polyphosphate granules and protein bodies accumulate in large numbers in cortical and medullary hyphae. Extra-cellular material is laid down very rapidly around hyphae of the cortex and medulla, until at maturity it almost completely fills any interhyphal spaces. The ultra-structure of young sclerotial hyphae is very similar to that of actively growing vegetative hyphae. The numbers of nuclei

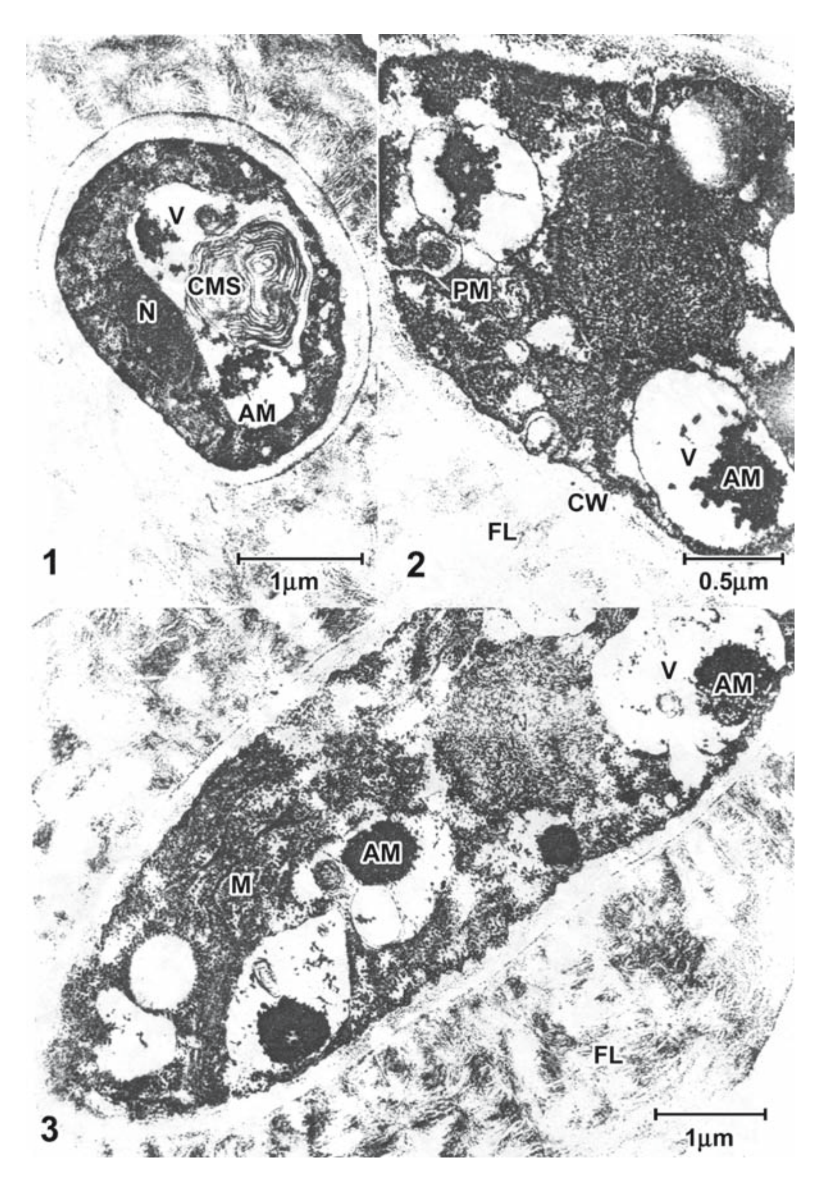


Plate 9.1.1.1.3 Ultra-thin sections of medullary cells of slightly pigmented sclerotium; (1) A large vacuole (V) including a concentric membranous structure (CMS) and electron-dense amorphous materials (AM); (2) Cell in process of thickening of wall. Note the significant invaginations of the plasma membrane and the deposition of electron dense, amorphous materials within vacuoles (V); (3) Cell including elongated mitochondria (M) and amorphous material containing vacuoles (V) (Adapted from the publication of Saito, 1977. With permission)

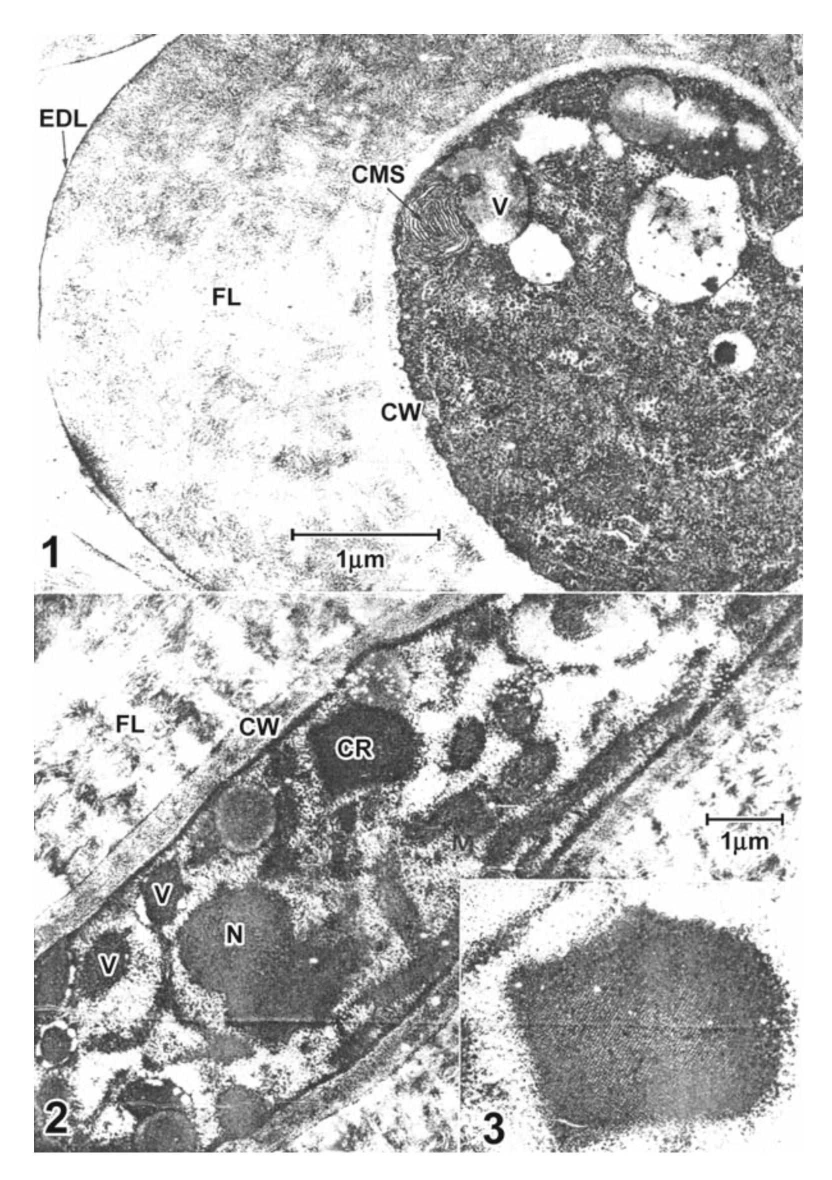


Plate 9.1.1.1.4 (1) Ultra-thin section of cell of slightly pigmented sclerotium showing a well-developed fibrous layer (FL) enveloped with a electron-dense thin layer (EDL). (2) Ultra-thin section of medullary cell of mature sclerotium showing degenerated mitochondria (M), vacuoles (V) filled with electron dense amorphous materials and a micro-body like inclusion having a crystalline structure (CR); (3) Enlarged inset of a micro-body like inclusion in 2 (Adapted from the publication of Saito, 1977. With permission)

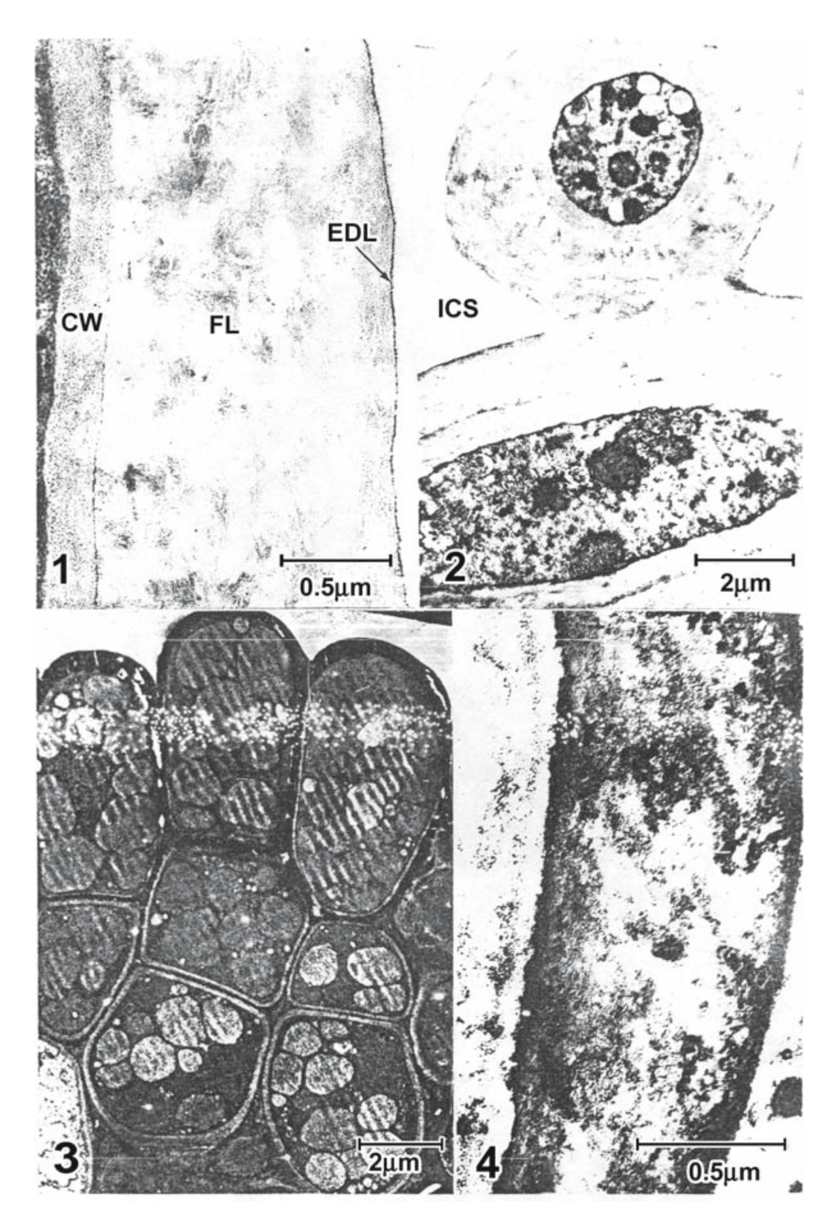


Plate 9.1.1.1.5 Ultra thin sections of mature sclerotium; (1) Portion of medullary cell wall showing the relative thickness of different layers; (2) A part of medulla showing intercellular space (ICS); (3) Rind layer; (4) Portion of rind cell wall showing large middle zone containing microfibrill and inner and outermost electron-dense layer (Adapted from the publication of Saito, 1977. With permission)



Plate 9.1.1.2.1 Ultra-thin sections of medullary cells of white sclerotium. (1) Invaginations (INV) of plasma membrane; (2) A transverse section of cell; (3) The in growth (arrows) of lateral wall to form a new septum; (4) Lomasomes (LO) located near the septum (S) (Adapted from the publication of Saito, 1977. With permission)

and profiles of mitochondria decrease at later stages of development but there is an increase in the number of profiles of endoplasmic reticulum cisternae. The cytoplasm has a granular appearance throughout differentiation. The general structure of mature sclerotia of *S. minor* is similar to that reported for sclerotia of other species in *Sclerotinia*.

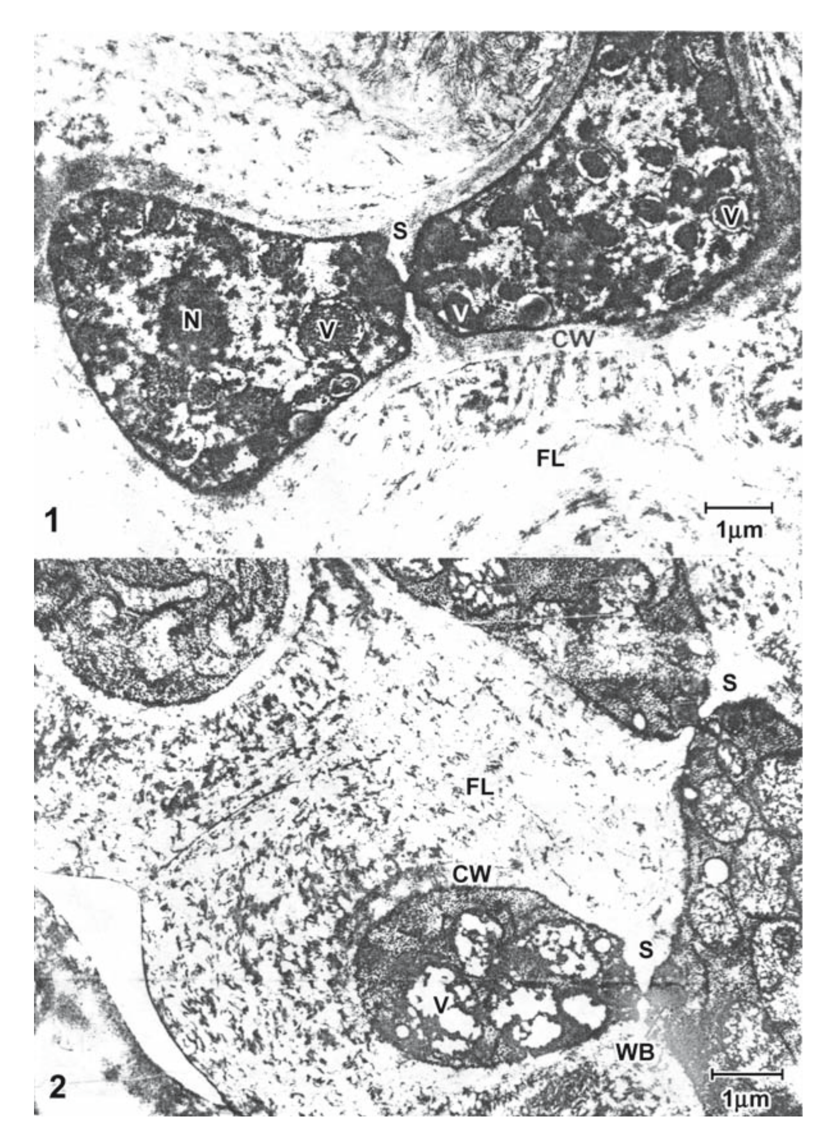


Plate 9.1.1.2.2 Ultra-thin sections of medullary cells of mature sclerotium varying fixation; (1) Cell having a septum, Glutaraldehyde + acrolein and O_8SO_4; (2) Cell having a septum, KM_nO_4 (Adapted from the publication of Saito, 1977. With permission)

9.1.2 Histochemistry of Sclerotia

The fibrous layer of *S. sclerotiorum* medullary cells have been found to be PAS (Periodic Acid Schiff Reaction) negative and dissolve completely in ca 23 M KOH after 2 h in the autoclave (Plate 9.1.2.1). Cell walls and septa are PAS positive and

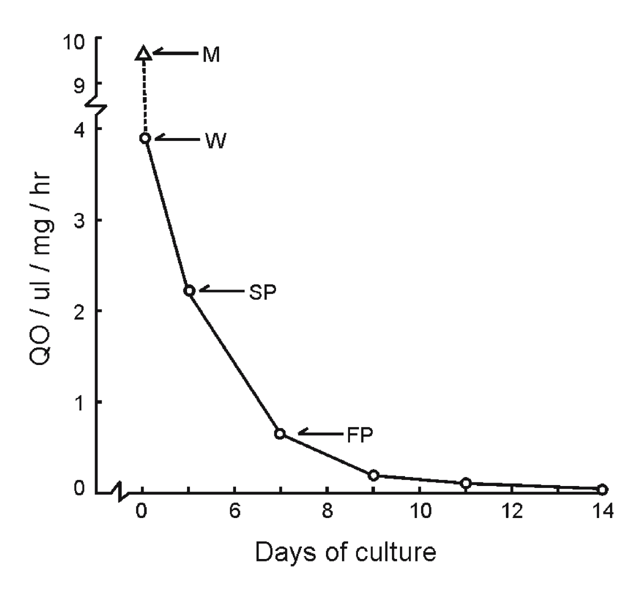


Fig. 9.1.1.2.1 Changes in the respiration rate of sclerotia during maturation (M: Mycelium; W: White sclerotium; SP: Slightly pigmented sclerotium; FP: Fully pigmented sclerotium (Adapted from the publication of Saito, 1977. With permission)

remain after autoclaving in the KOH solution. In the remaining cell walls and septa, chitosan is detected using I Ki in 1 per cent H_2SO_4. Under ultraviolet light, yellow fluorescence is seen in both the cell wall and the fibrous layer, if cells are stained with dilute aniline blue. This suggests the inclusion of β-1, 3 glucans in both layers despite the faint fluorescence in the latter. Medullary cells are subjected to digestion by partially purified β-1, 3 glucanase with or without papain (Plate 9.1.2.2). It has been found that incomplete lysis of these layers occurs only by the combination of the two enzymes. From these results, it is concluded that the walls of medullary cells contain chitin, β-1, 3 glucans and protein components (Jones, 1970; Saito, 1974a, 1977). The fibrous layer has β-1, 3 glucans and proteins as components (Saito, 1974a, 1977).

The cytoplasm of medullary cells of mature sclerotia of *S. sclerotiorum* has been found to contain PAS positive materials (Plate 9.1.2.1). Since the PAS stainability is reduced or occasionally disappears after an amylase digestion of cells, such materials are considered to be glycogen (Plate 9.1.2.1). In mature sclerotia, the medullary cells contain many granules which increase in number as sclerotia mature and probably correspond to vacuoles containing electron-dense, amorphous materials suggesting accumulation of polyphosphates in vacuoles of mature sclerotia (Saito, 1977).

The composition, cellular form and distribution of storage reserves within sclerotia of *S. minor* have been determined by Bullock et al. (1980b) using histochemical techniques to complement the detailed ultra-structural study on the development of these sclerotia described earlier. The walls and septa of all hyphae contain chitin and β-1, 3 glucans, while those of the rind contain in addition, a melanin-like

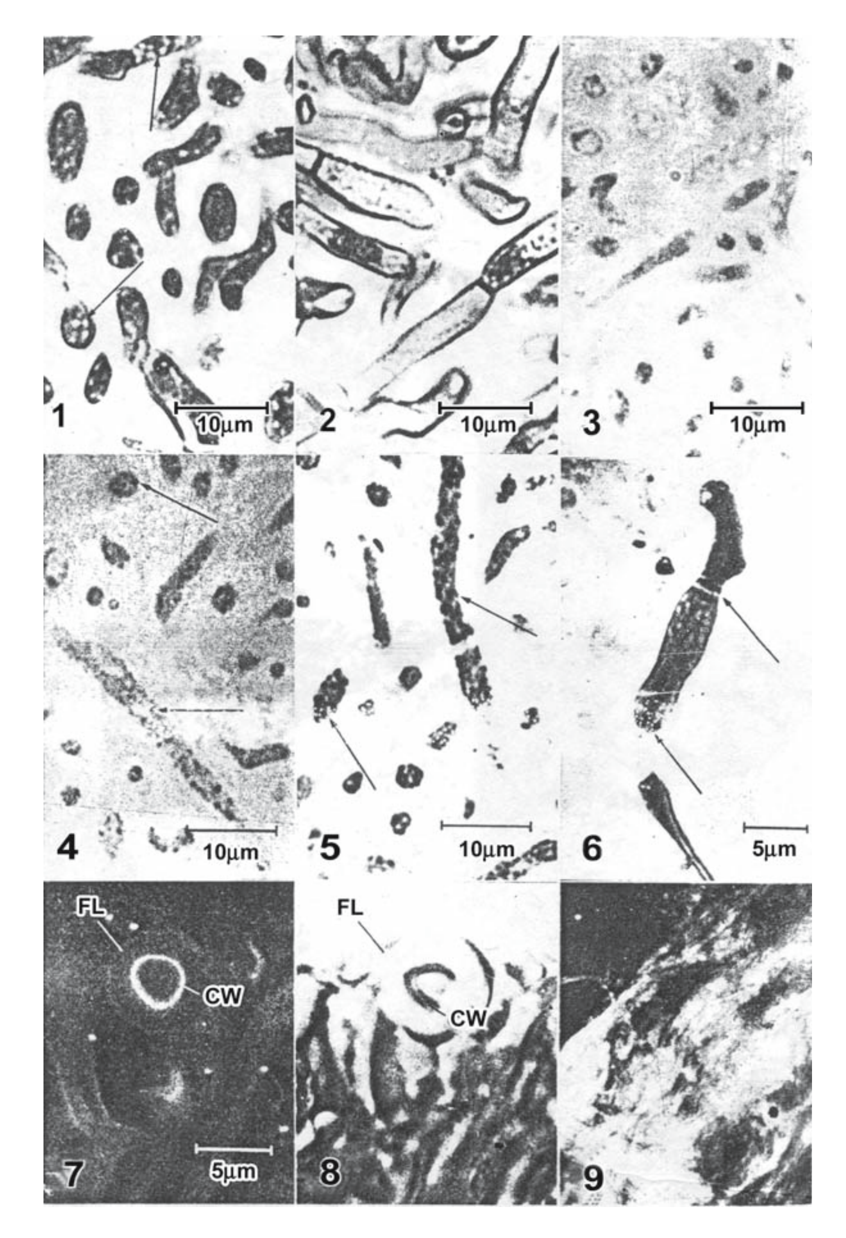


Plate 9.1.2.1 (1) Untreated section of mature sclerotium stained with PAS reagent, Arrows indicate PAS negative granules; (2) Section of the same tissue subjected to the α-amylase digestion; (3) HPMA section of white sclerotium stained with aniline blue, pH 4.4; (4) Same section of slightly pigmented sclerotium; (5) Same section of mature sclerotium. Note the metachromatic granules (arrows); (6) Chitosan reaction in cell wall and septa (arrows) of medullary cell of mature sclerotium; (7) A fluorescence micrograph of medullary cells stained with diluted aniline blue, pH 9.0; (8) Same micrograph as (7) Except for ordinary illumination; (9) A fluorescence micrograph of isolated β-1, 3 glucans stained with diluted aniline blue (Adapted from the publication of Saito, 1977. With permission)



Plate 9.1.2.2 Enzyme treated sections of mature sclerotium; 1–3 stained with PAS; 4–6, unstained, phase contract; (1) Untreated section; (2) Section treated with β-1, 3 glucanase; (3) Section treated with β-1, 3 glucanase + papain; (4) Untreated section; (5) Section treated with β-1, 3 glucanase; (6) Section treated with β-1, 3 glucanase + papain (Adapted from the publication of Saito, 1977. With permission)

pigment. An extra-cellular matrix, which accumulates around cortical and medullary hyphae, consists primarily of β-1, 3 glucans, although another polysaccharide, which could not be identified by histochemical methods is also present. Phenolic material is deposited around the extra-cellular matrix and in the few inter-hyphal spaces that remain at maturity. Glycogen is present throughout the cytoplasm of hyphae of the cortex and medulla, at all stages of their differentiation.

Polyphosphate granules are laid down within small vacuoles and as sclerotia mature, become most common in the cortical region. Protein bodies develop rapidly in cortical and medullary hyphae until at maturity, they are the most obvious inter-hyphal feature. These bodies are round or elongated in shape, the elongated ones often lying parallel to the long axis of the hyphae and in close association with strands of endoplasmic reticulum. No lipid reserves are detected.

9.1.3 Histology of Normal and Abnormal Sclerotia

The occurrence of morphologically abnormal sclerotia of *S. sclerotiorum* collected from diseased sunflower plants have been reported (Huang, 1982). In contrast to the normal sclerotia which have a relatively smooth surface and a white medulla, the abnormal ones are rough in appearance and the center of the medullary tissue is brown. The sclerotial abnormality is not heritable. Nevertheless, variability and pathogenicity of abnormal sclerotia are decreased compared with normal sclerotia (Huang, 1982). The histological study indicated that normal sclerotia of *S. sclerotiorum* from diseased sunflower tissue consist of three distinct layers (rind, cortex and medulla) and the detailed structure of these layers is similar to that described earlier (Kosasih and Willetts, 1975; Saito, 1977). The normal sclerotia contain a relatively intact ring layer two or three cells wide, cortex two to four cells wide and a large white medullary region with numerous darkly stained, loosely arranged interwoven hyphae embedded in an amorphous matrix. In contrast, the abnormal sclerotium has a severely fractured rind and a brown medullary region with sparse, lightly stained filamentous hyphae embedded in the amorphous matrix which is often highly vacuolated. The viable cells in abnormal sclerotia are mainly confined to the white medullary region and there is little evidence of hyphal growth from the brown coloured medullary tissue. The leakage of amino acids is greater in the abnormal sclerotia than in normal ones. Chemical analyses revealed that protein, alcohol-soluble substances and oil contents are similar in normal and abnormal sclerotia (Table 9.1.3.1), but the amount of ash is significantly higher in the abnormal ones (Huang, 1983). The normal sclerotia of *S. sclerotiorum* from diseased sunflower plants have percentage of oil, protein and alcohol soluble substances comparable with those from diseased pea or bean plants (LeTourneau, 1966; Weete et al., 1970). However, the linoleic acid in sclerotia from diseased sunflower plants appears to be higher than from diseased pea plants (Weete et al., 1970).

9.2 Sclerotial Germination

Carpogenic germination of a sclerotium of *S. sclerotiorum* is apparent when an apothecial stipe protrudes from the sclerotium. Four stages are involved in stipe genesis (Plates 8.2.1, 9.2.1). The first evidence of primordium formation is detected

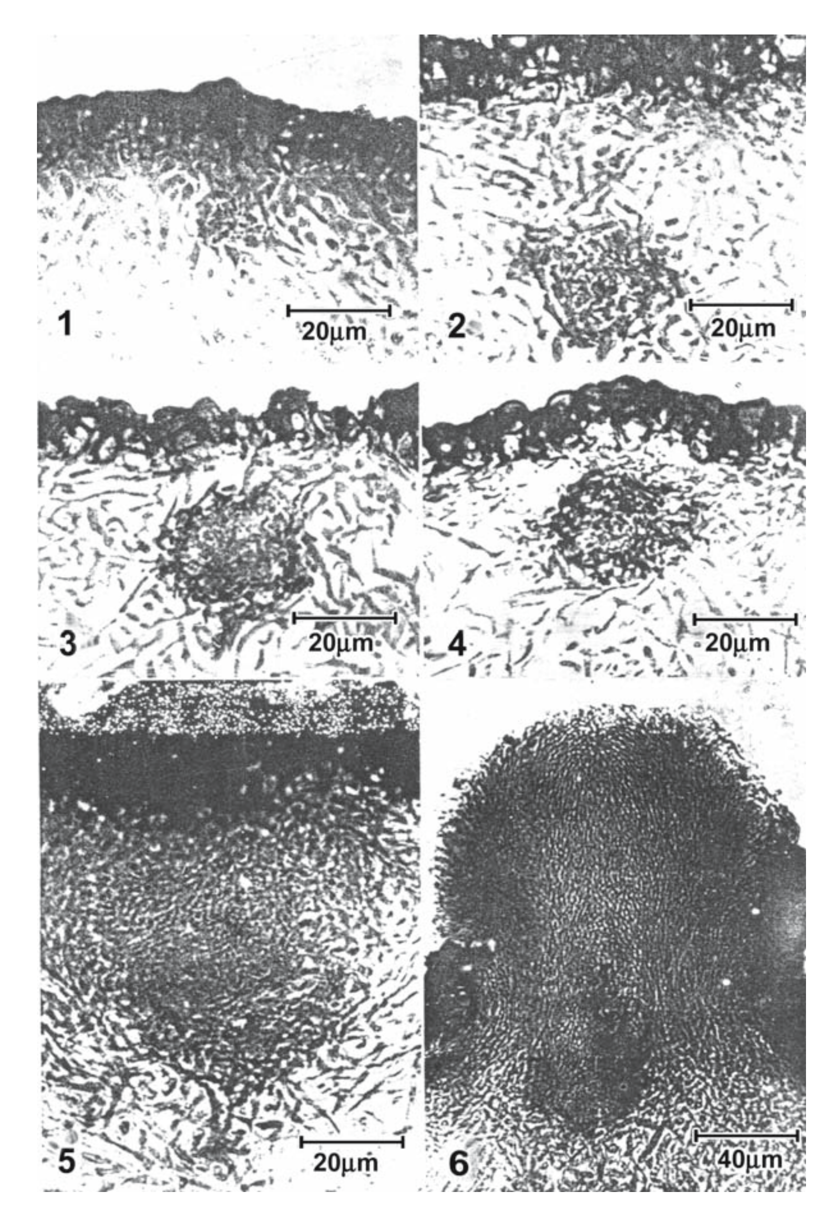


Plate 9.2.1 Vertical sections of sclerotium showing successive stages of apothecial stipe development; (1) Stage I – primordium showing deeply stained meristematic structure; (2) Stage I – primordium increased in size, but pigmentation not yet occurs; (3) Stage II – primordium. Dark pigments occur around the primordium; (4) Stage-II – Primordium infiltrated with pigmentation; (5) Stage III – primordium; (6) Stage IV – primordium (Adapted from the publication of Saito, 1977. With permission)

Table 9.1.3.1 Chemical components of normal and abnormal sclerotia of *Sclerotinia sclerotiorum* from sunflower heads (Adapted from the publication of Huang, 1983. With permission)

	Sclerotia	
Chemical components[a]	Normal	Abnormal
Oil	1.02	0.92
Protein	12.8	13.1
Alcohol-soluble materials	12.1	12.1
Ash[b]	1.7	2.1
Fatty acid		
Palmitic C 16:0	13.1	13.8
Stearic C 18:0	1.8	1.7
Poleic C 18:1	22.6	19.1
Linoleic C 18:2	53.2	50.9
Linolenic C 18:3	8.2	12.0

[a]Values of oil, protein, alcohol-soluble material and ash are percentages of the dry weight matter and values of fatty acids are percentages of the total oil.
[b]Significant difference in the amount of ash between normal and abnormal sclerotia ($P < 0.05$). There were no significant differences between values of the other components.

as deeply stained areas in the medulla near the rind of the sclerotium. These areas are composed of both narrow and somewhat broader, thin-walled cells with dense cytoplasm (Stage I). Subsequently, dark pigments develop around or inside the primordia (Stage II), this seems to result from the formation of darkly pigmented, thick-walled cells. A mass of thin-walled hyphae with dense cytoplasm arise from the stage II primordia and develop into a tissue having a structure which is clearly distinguished from the medulla beneath the rind of sclerotia (Stage III). Primordia rupture the rind and begin to grow as young apothecial stipes (Stage IV). Small cubes of medullary tissue obtained from mature sclerotia germinate after the surface becomes darkly pigmented or after the rind regenerate, subsequently produces normal apothecia (Plate 9.2.2). In such medullary tissue cubes, primordia are also initiated near the regenerated rind. These results indicate that any part of the medulla has the potential for primordium initiation and that the location of the initiation closely relates to the site of rind differentiation (Saito, 1977).

9.2.1 Ultra-structures

In most of the medullary cells of germinating sclerotia, vacuoles filled with polyphosphate-like substances are still prominent in the cytoplasm and mitochondrial degeneration is still evident (Plate 9.2.1.1). Thus, there is no significant difference between metabolic activities in most of the medullary cells of germinating sclerotia and those of matured but ungerminating sclerotia. However, a remarkable development

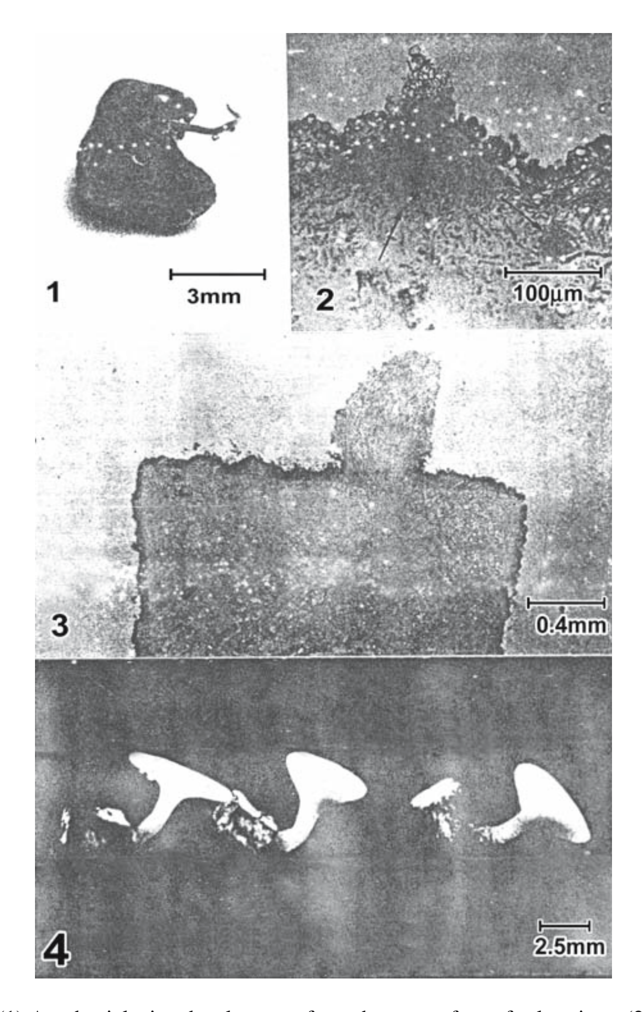


Plate 9.2.2 (1) Apothecial stipe development from the cut surface of sclerotium; (2) Section of a cubed medullary tissue with regenerated rind producing apothecial stipe primordia (arrows); (3) Section of a cubed medullary tissue showing regenerated rind and its germination; (4) Development of mature apothecia from cubed medullary tissues (Adapted from the publication of Saito, 1977. With permission)

of endoplasmic reticulum and zonation of cell wall are characteristically seen in the former. On the other hand, the cells composing the stipe primordia are easily distinguished from such undifferentiated medullary cells (Plate 9.2.1.2-Fig. 3, Plate 9.2.1.3). The primordial cells are quite irregular in their shape and size and have thin homogenous walls lacking fibrous layers. More characteristically there are many ribosomes and mitochondria with distinctive cristae reflecting their high

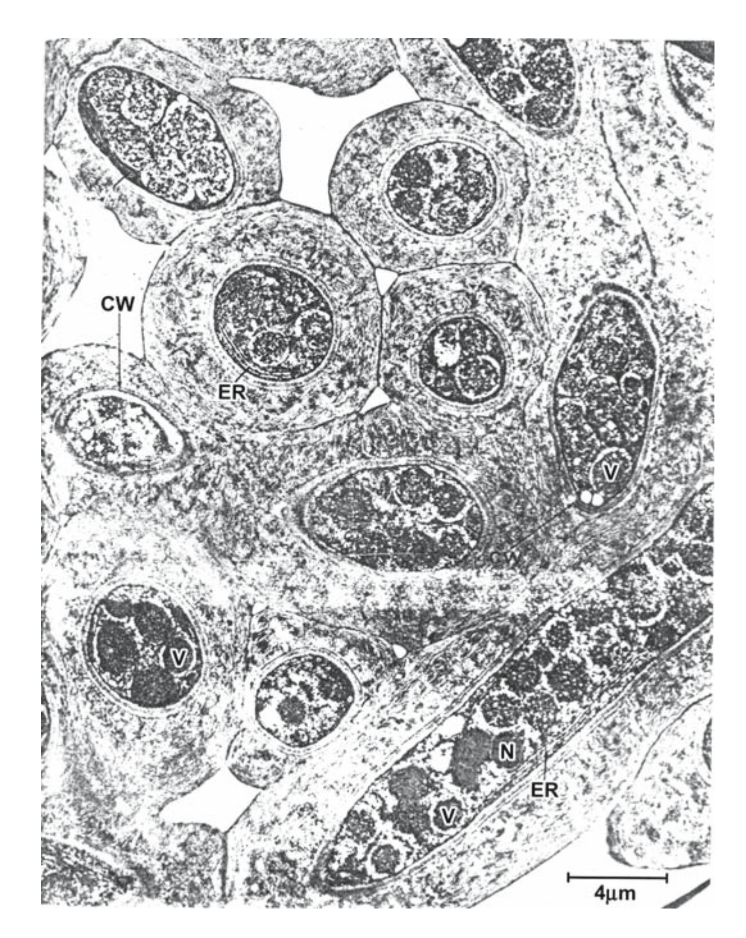


Plate 9.2.1.1 Ultra-thin section of germinating sclerotium showing the cellular condition of non-primordial region in medulla. Vacuoles (V) filled with electron-dense, amorphous materials are predominating in cytoplasm, but well-developed endoplasmic reticulum (ER) zonation of cell wall (CW) are seen (Adapted from the publication of Saito, 1977. With permission)

metabolic activities (Plate 9.2.1.4). Polyphosphate-like substances are not seen in vacuoles of primordial cells. Such substances are found to decrease in volume in the vacuoles of adjoining medullary cells, suggesting their utilization as energy sources. Stage II -like primordia with electron dense deposits are occasionally observed (Plate 9.2.1.5). However, such deposition occurs not in the cell walls but in the intercellular matrix of the primordia. It appears in fibrous materials remaining among cells or also in the fibrous layer of adjoining medullary cells. Thus, pigmentation observed might have originated in fibrous materials, presumably from their melanization (Saito, 1977).

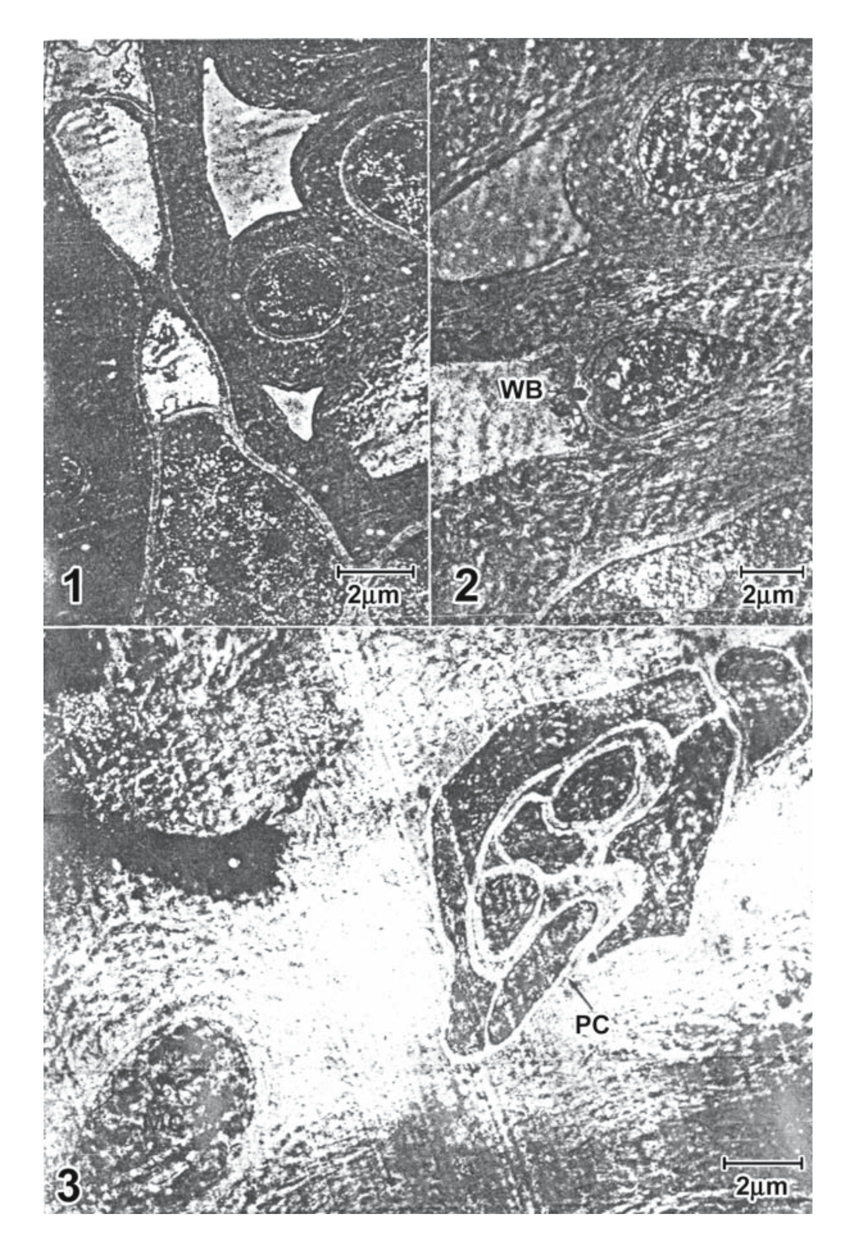


Plate 9.2.1.2 (1) Degenerated cell in medulla of germinating sclerotia; (2) Almost completely degenerated cell adjacent to healthy one, A pore is plugged with a Woronin body (WB); (3) A primordial cell cluster in medulla. Note the difference of size between primordial cells (PC) and medullary cells (MC) (Adapted from the publication of Saito, 1977. With permission)



Plate 9.2.1.3 Ultra-thin section of medullary tissue showing a contrasted appearance of primordial cells (PC) and medullary cells (MC). Note the decrease of contents in vacuoles (V) in medullary cells adjoining to primordial and deposition of electron-dense materials among primordial cells (Adapted from the publication of Saito, 1977. With permission)

The primordial cells, thin-walled and lacking fibrous layers, can be differentiated from medullary cells. Direct outgrowths of medullary cells could not be found. However, there are some endo-hyphae like cells which appear to be produced by separation of two different layers occurring in the thickened wall of medullary cells (Plate 9.2.1.6). Such endohyphae like cells seem to have higher metabolic activities than undifferentiated medullary cells, because they have many ribosomes and mitochondria in the cytoplasm. The development of endo-hyphae like cells is the initial



Plate 9.2.1.4 (1) A part of primordium; (2) Cytoplasmic appearance of a primordial cell showing many ribosomes and mitochondria (Adapted from the publication of Saito, 1977. With permission)

step of cellular differentiation. Cells thus grow from inert medullary cells, lysing their mother cell wall and the fibrous layer. The zonation in medullary cell walls mentioned earlier is a prerequisite for cellular differentiation, and in turn β-1, 3 glucanase also functions in such processes. As in many fungi, endo hyphae occur in a dead cell by cell protrusion through the septal pore from an adjoining living cell. Consequently, the above-mentioned situation may be termed "ecdysis" of cells

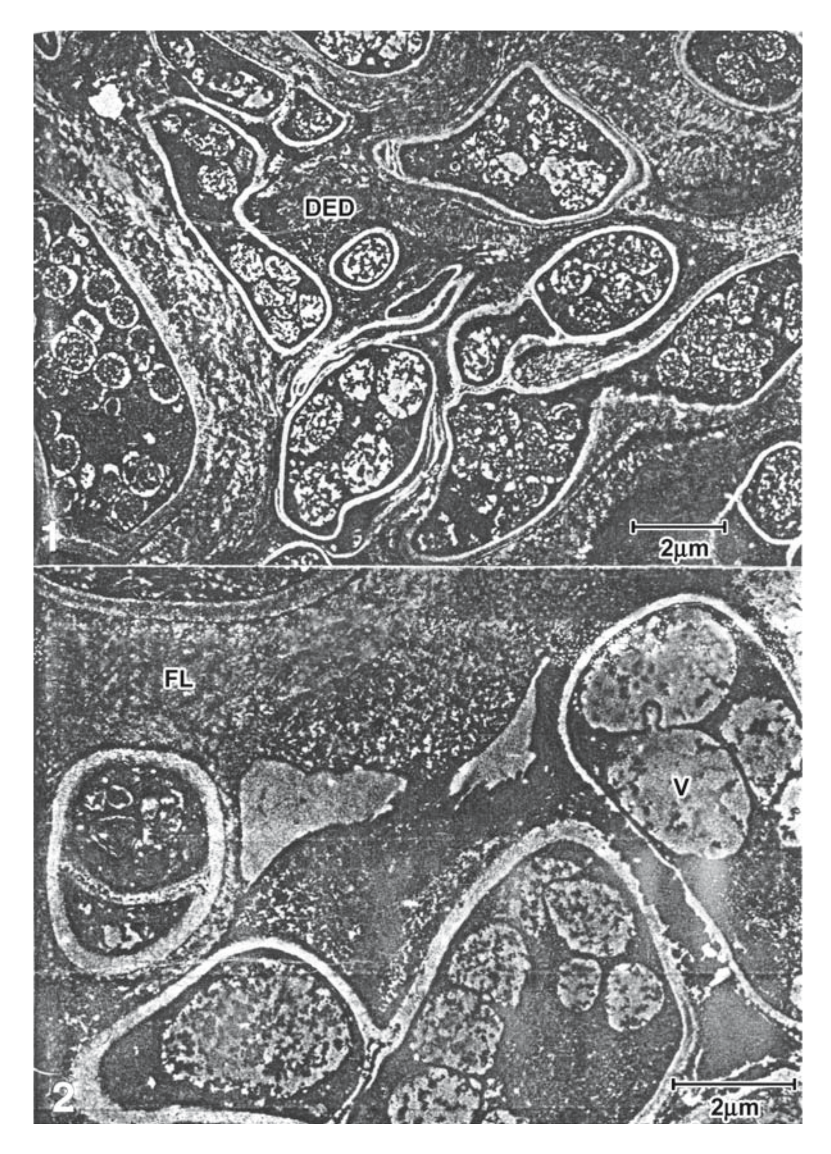


Plate 9.2.1.5 (1) Ultra-thin section of a primordium. Note deposition of electron dense materials (DED), and thin wall, irregular shape and size of primordial cells; (2) Peripheral part of primordium showing deposition of electron dense materials un fibrous layer of adjoining medullary cells (Adapted from the publication of Saito, 1977. With permission)

rather than endo-hyphae formation. In the medulla of decayed sclerotia, cell walls disappear and fibrous layers decompose (Plate 9.2.1.7) indicating the consumption of component materials such as β-1–3 glucans or proteins of such cells during apothecial production. However, fibrous layers are sometimes seen to be intact. Numerous mitochondria and ribosomes are observed in the cells constituting stipe

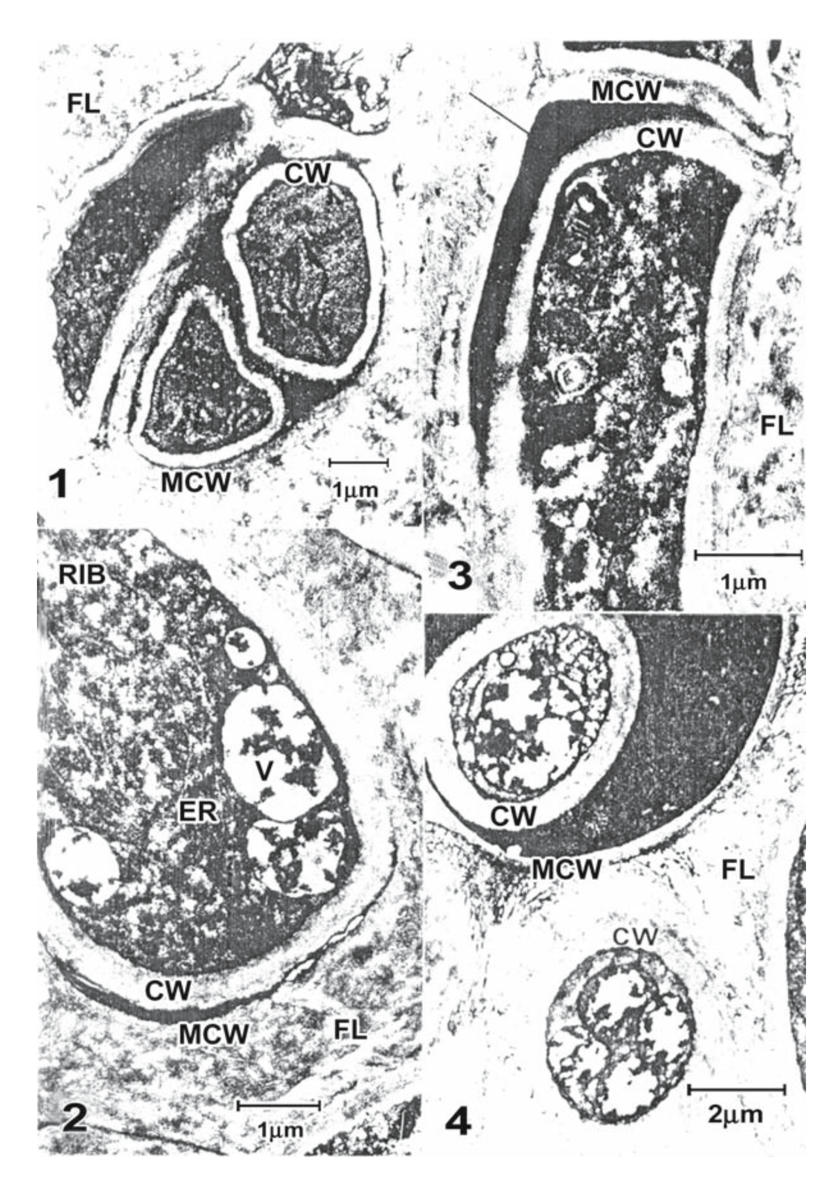


Plate 9.2.1.6 (1) Endo-hyphae like cells in medulla. $KMnO_4$; (2) Ultra-thin section of a medullary cell of which different layers in a cell wall are separated each other. Note many ribosome and endoplasmic reticulum; (3) Ultra-thin section of medullary cell at the same state of (2). Note highly electron-dense zone between the separating layers; (4) Endo-hyphae like cell in the medulla of germinating sclerotia of *Sclerotinia borealis* (Adapted from the publication of Saito, 1977. With permission)

tissue, indicative of the active synthesis of ribonucleic acids and high metabolic activities (Plate 9.2.1.8) in such cells (Saito, 1977). According to Bullock and Willetts (1996) during mycelial germination of sclerotia of *S. minor* germ hyphae, which are initiated in the outer regimes of the sclerotium, pass through degenerating

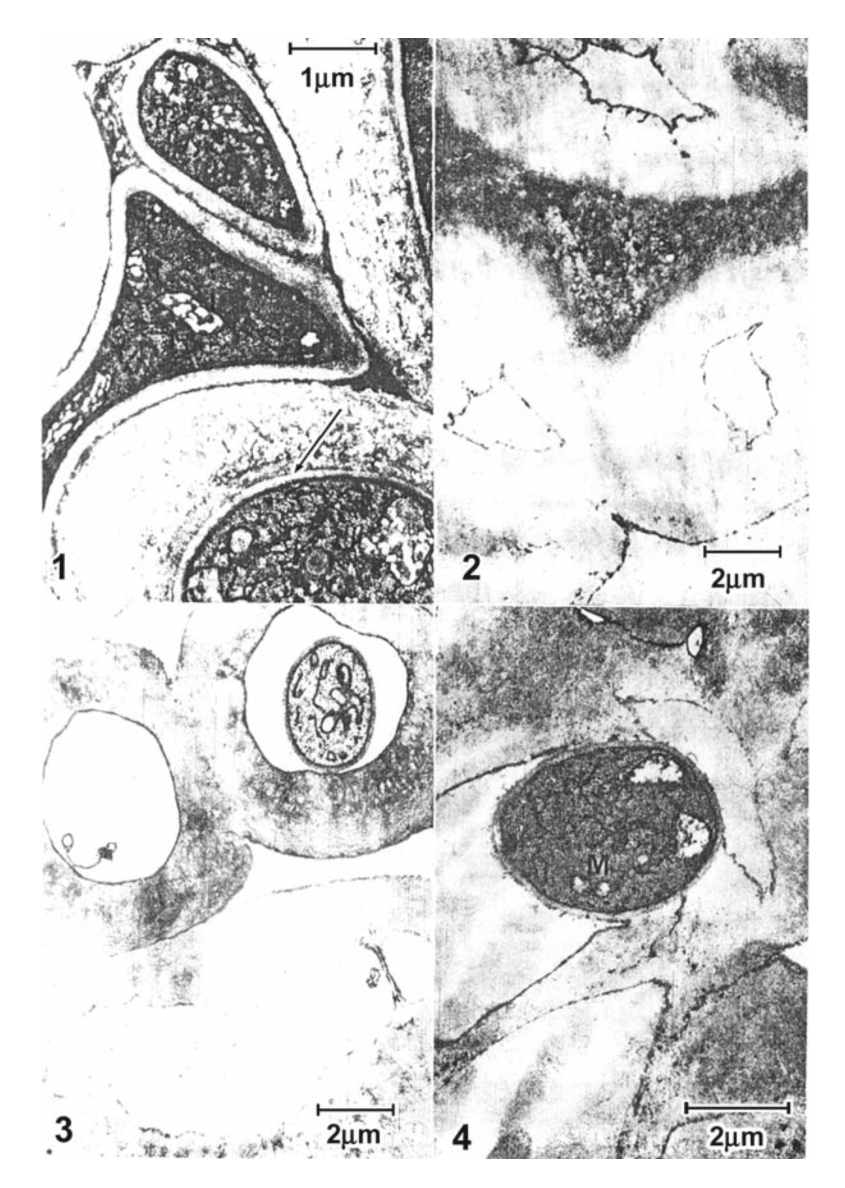


Plate 9.2.1.7 (1) Distorted cells of stipe fundament in medullary cells. Zonation occurs in medullary cell wall (arrow) $KMnO_{4.}$; (2) A part of medullary tissue of a decayed sclerotium from which many apothecial have been produced. Cell walls disappear and fibrous layers mostly lose the structure, $KMnO_4$; (3) Fibrous layers holding their structure in decayed medullary tissue, $KMnO_{4.}$; (4) A transverse section of secondarily formed hyphal cell in decayed medullary tissue, $KMnO_4$ (Adapted from the publication of Saito, 1977. With permission)

outer medullary. The cortical hyphae emerge individually through the rind and aggregate outside the sclerotium to form a mycelium. This type of mycelial germination is described as ‘non-eruptive’. Almost all intra and extracellular sclerotial reserves are utilized in the production of germ hyphae.



Plate 9.2.1.8 Ultra thin sections of cells composing stipe tissue; (1) a cell located in basel region of stipe. Note the zonation (arrow) of cell wall (CW) many mitochondria (M) and ribosomes in cytoplasm (Adapted from the publication of Saito, 1977. With permission)

9.2.2 Histochemistry

An intensive PAS is detected in stipe primordia (Plate 9.2.2.1). Protein concentration is not high in primordia at stage I as indicated by mercuric bromophenol blue and the Millon reaction. However, the reaction becomes more intense in primordia as they develop and is most intense in the apical regions of young growing stipes (Plates

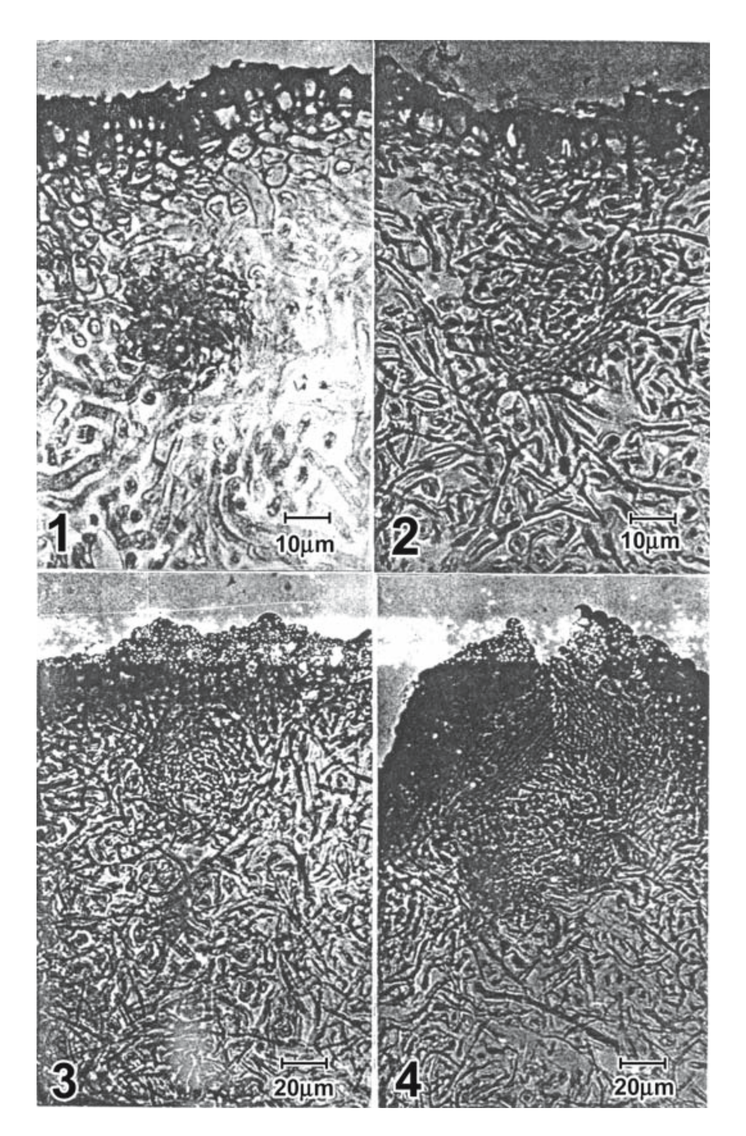


Plate 9.2.2.1 Light micrographs of histochemical reaction in sections of sclerotium; (1) PAS reaction in a primordium and the surrounding medullary tissue; (2) Proteins stained with mercuric bromophenol blue in a primordium and surrounding medullary tissue; (3) The same staining as (2); (4) the same as (2). A primordium at later phase of stage III (Adapted from the publication of Saito, 1977. With permission)

9.2.2.1, 9.2.2.2). There is a remarkable difference of RNA content between primordial and nonprimordial regions of the medulla indicating the active synthesis of RNA in primordial cells. There are intense black depositions in primordia, especially in those pigmented (Stage II) as well as rind cells (Plate 9.2.2.3). Phenolic compounds are localized in primordia and their pigmentation at stage II results from melanin

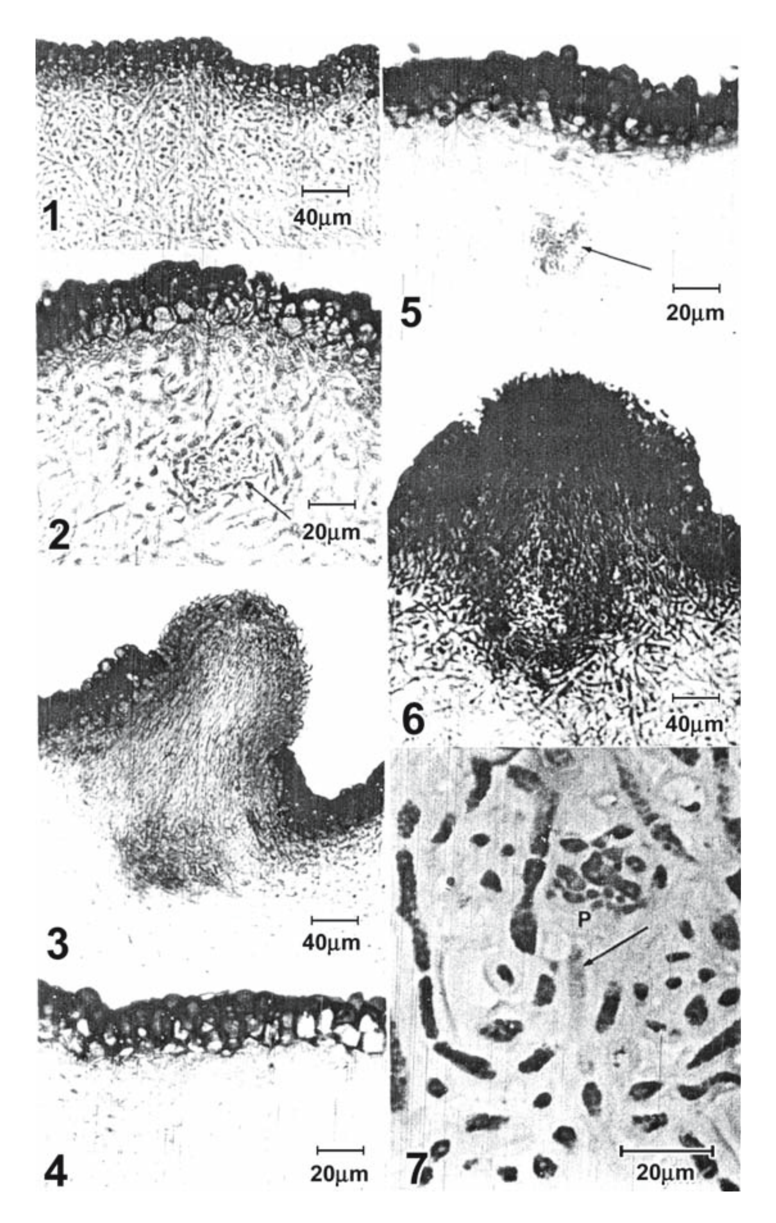


Plate 9.2.2.2 Light micrographs of histochemical reaction in sections of sclerotium; (1) Millon reaction in non-primordial region of medullary tissue; (2) Millon reaction in a primordium and the surrounding medullary tissue; (3) Millon reaction in a state IV-primordium; (4) Non-primordial region of medullary tissue stained with pyronin; (5) Pyronin staining of a primordium and surrounding medullary tissue. An intense staining in primordium (arrow); (6) Stage IV– primordium stained with toluidine blue; (7) HPMA section stained with toluidine blue. A primordium (P) is stained blue and lacking polyphosphate like granules. An adjoining medullary cell lacking such granules is seen (arrow) (Adapted from the publication of Saito, 1977. With permission)

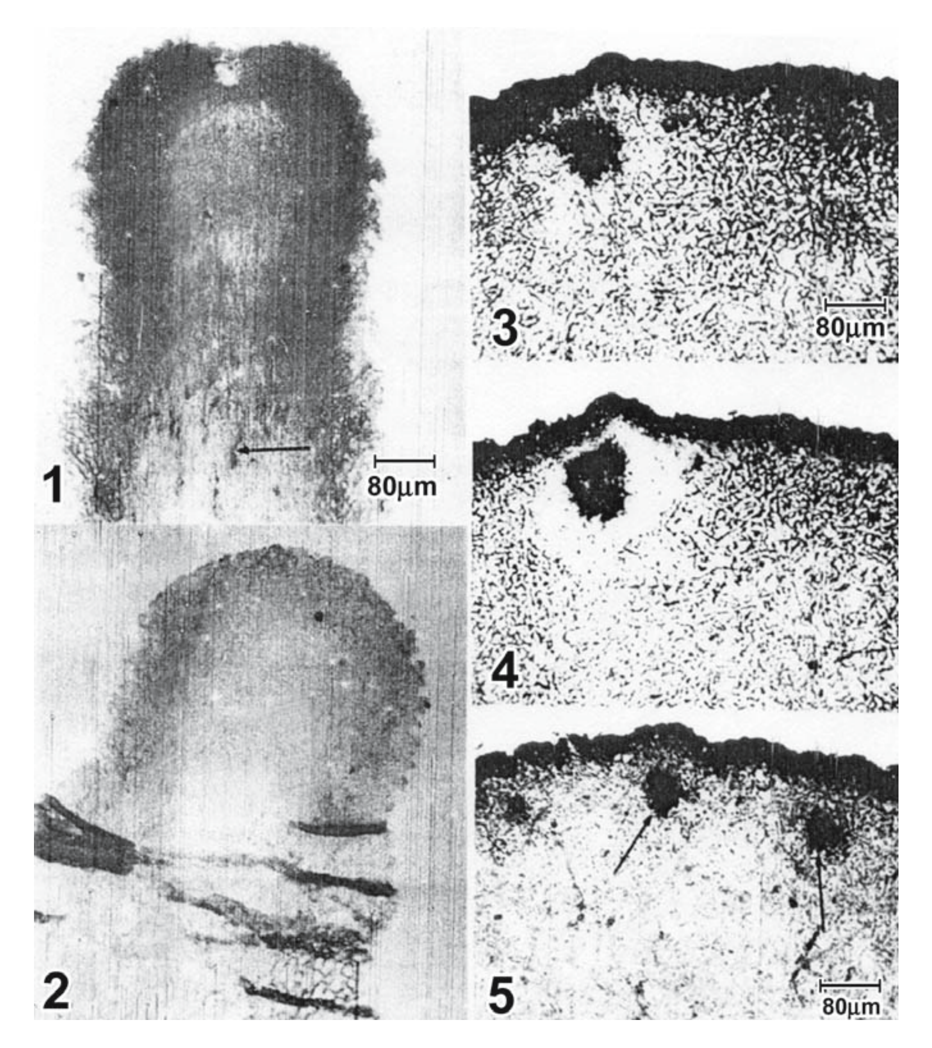


Plate 9.2.2.3 (1) Section of young apothecial stipe stained with pyronin. Intense staining is seen in apical region and some cells distributing in middle zone (arrow); (2) Same section treated with ribonucrease and stained as (1); (3) Section of sclerotia including a stage II-primordium stained with toluidine blue; (4) Same section treated with ribonucrease and stained as (3); (5) Section stained with Masson's ammonical silver nitrate. Intense staining is seen in primordial (arrows) as well as rind (Adapted from the publication of Saito, 1977. With permission)

formation from the oxidative polymerization of poly phenols. Phenolic compounds are probably present in fibrous materials. Succinate dehydrogenase activity was histochemically investigated using nitro blue tetrazolium (Plate 9.2.2.4). Blue black depositions of formazan are detected in the cells composing the central region of young apothecial stipes. However, no detectable activity is observed in the non primordial region in the medulla of germinating sclerotia (Saito, 1977).

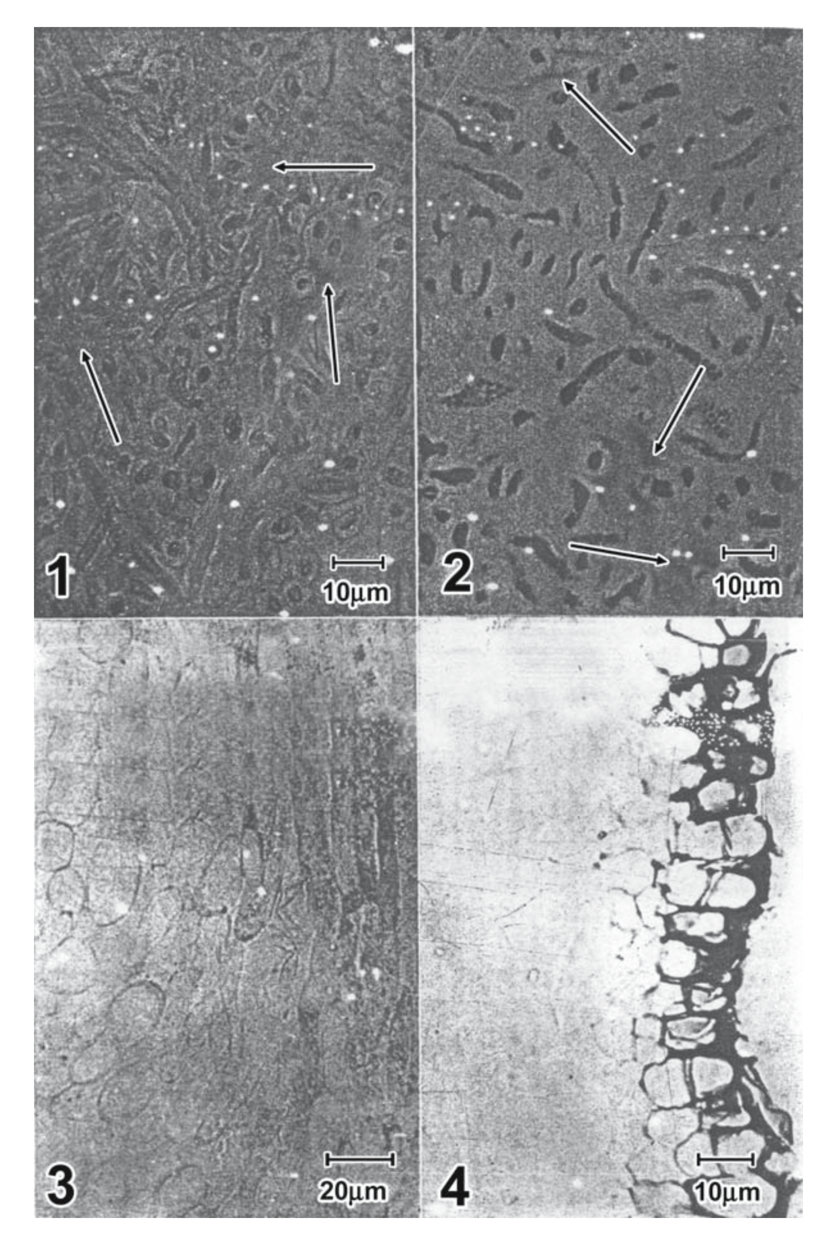


Plate 9.2.2.4 (1) Section of medullary tissue of germinating sclerotia showing basophilic, intercellular matrix in pyronin staining (arrows); (2) Basophilic, intercellular matrix in toluidine blue staining (arrows); (3) Distribution of succinate dehydrogenises activities in longitudinal section of young stipe. Note high activities in the cells of inner part (right); (4) The same reaction as (3) in the non-primordial region of medulla of a germinating sclerotium (Adapted from the publication of Saito, 1977. With permission)

9.2.3 Ultra-structure of Stipe and Apothecium

The germinating sclerotia of *S. sclerotiorum* after eight to ten weeks incubation in pots at 20°C are shown in Plate 9.2.3.1-Fig. 1. Some sclerotia bear more than one stipe and apothecia in different stages of development can be seen. The stipe is seen to be made up of a bundle of longitudinally arranged, closely packed hyphae (Plate 9.2.3.1-Fig. 2). The immature apothecia (Plate 9.2.3.1-Fig. 3) with undifferentiated hyphae (Plate 9.2.3.1-Fig. 4) develop into a trumpet-shaped structure (Plate 9.2.3.1-Fig. 5) in which asci with inoperculate apices (Plate 9.2.3.1-Fig. 6) occur. A longitudinal section through an ascus, with unicellular ascospores (only six out of eight shown) and through one from which ascospores have been discharged is illustrated (Plate 9.2.3.2-Fig. 7). Since this empty ascus has probably been sectioned obliquely, the aperture (Plate 9.2.3.2-Fig. 8) through which the spores are ejected, is not present. In the immature ascus, nuclei with characteristic two-layered membranes are evident (Plate 9.2.3-Fig. 9). The walls delimiting the ascospores are formed later (Plate 9.2.3.2-Fig. 10). Sometimes more than one nucleus is found in the mature ascospores (Plate 9.2.3.2-Fig. 11). Various unidentified inclusions as well as vacuoles are to be found in the ascospore (Plate 9.2.3.2-Fig. 12). Similar vacuoles can also be seen in the ascus cavity surrounding the ascospores. Nuclei can be readily identified in freeze-etched ascospores by the presence of pores in the nuclear membrane. These pores can be seen in Plate 9.2.3.3-Fig.1, where the nucleus is fractured in such a way that the outer surface of the membrane is exposed and in Plate 9.2.3.3-Fig.1, spore 4, where the inner surface is revealed. The pores also appear as gaps in the nuclear membrane of cross fractured nuclei (Plate 9.2.3.2-Fig. 8). Vacuoles can be identified in freeze-etched ascospores (Plate 9.2.3.2-Fig. 9) fractured with the outer surface of the vacuolar membrane exposed in some and the inner surface in others. Plate 9.2.3.2-Fig. 10, inset, illustrates the inner surface of the plasmalemma with characteristic ridges, which appear as invaginations when the outer surface is exposed. A composite photograph of a fractured and etched ascus with ascospores (seven out of the eight shown) is given in Plate 9.2.3.2-Fig. 8, the fracture has occurred in such a way that the different surfaces of the various spore organelles described above are revealed. Various other structures, possibly immature asci and paraphyses are evident in the fractured apothecium. Figure presented in Plate 9.2.3.3 shows a semi diagrammatic representation of the fractured ascus. The IR spectra of stipe and apothecial walls closely resembles that of the hyphal walls of *S. sclerotiorum*, in which chitin and β-1–3, glucan are both present and where the chitin forms a considerable proportion of the wall components (Jones, 1970, 1974a).

The ascus vesicle, the muff-like open ended membrane system that carves out sporogenous area in ascus development has been found to give a distinctly stronger reaction for polysaccharides, than the plasmalemma of the ascus which the component membranes of that system otherwise closely resemble. Striking changes in the amount and distribution of stored glycogen and in the organization of the cytoplasm of developing *Sclerotinia* asci are described as also carpogamy, development of the complex apical apparatus and ascospore walls (Codron, 1974).

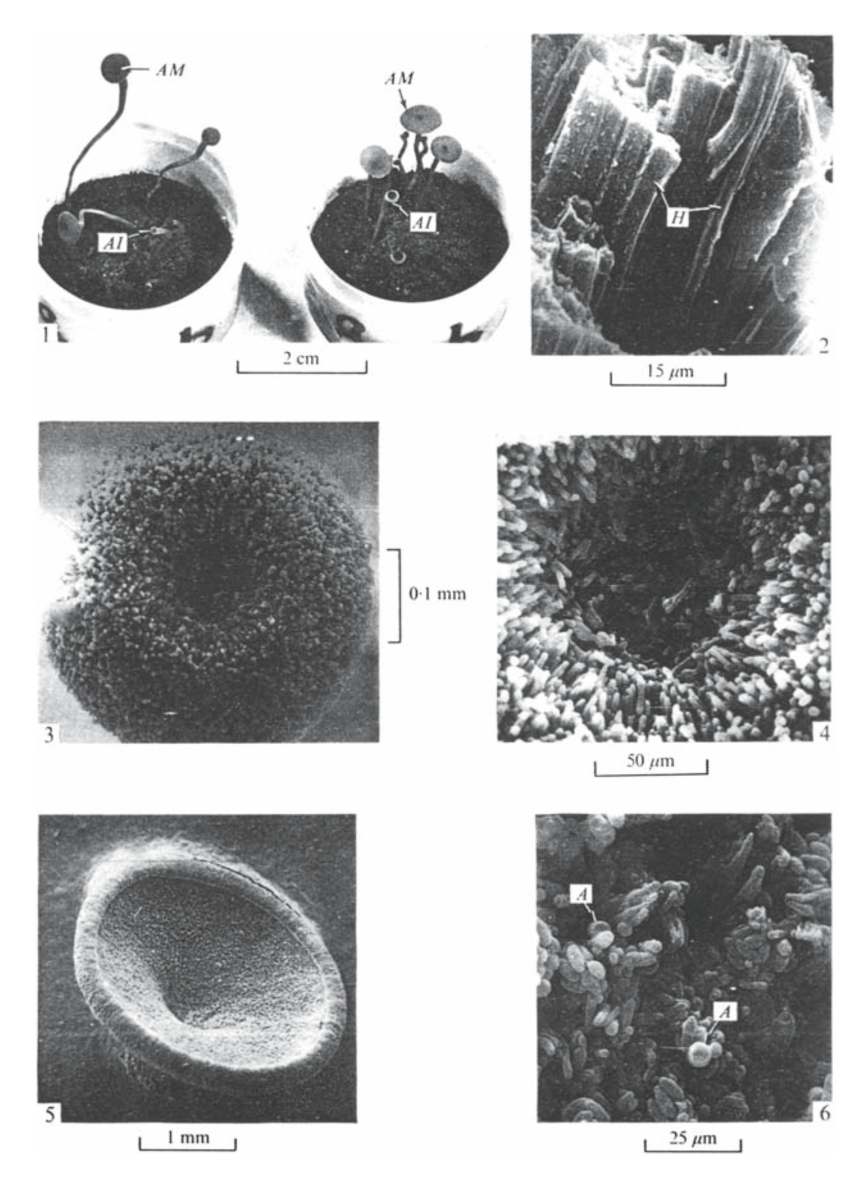


Plate 9.2.3.1 (1) Germinating sclerotia on soil, in pots, with stipes and apothecia in different stages of development; (2–6) Scanning electron micrographs of severed stipe (Fig. 2), Immature apothecium (Figs. 3 and 4) and mature apothecium (Figs. 5 and 6). A: Asci; Al: Immature apothecium; AM: Mature apothecium; H: Hyphal strands (Adapted from the publication of Jones, 1974a. With permission)

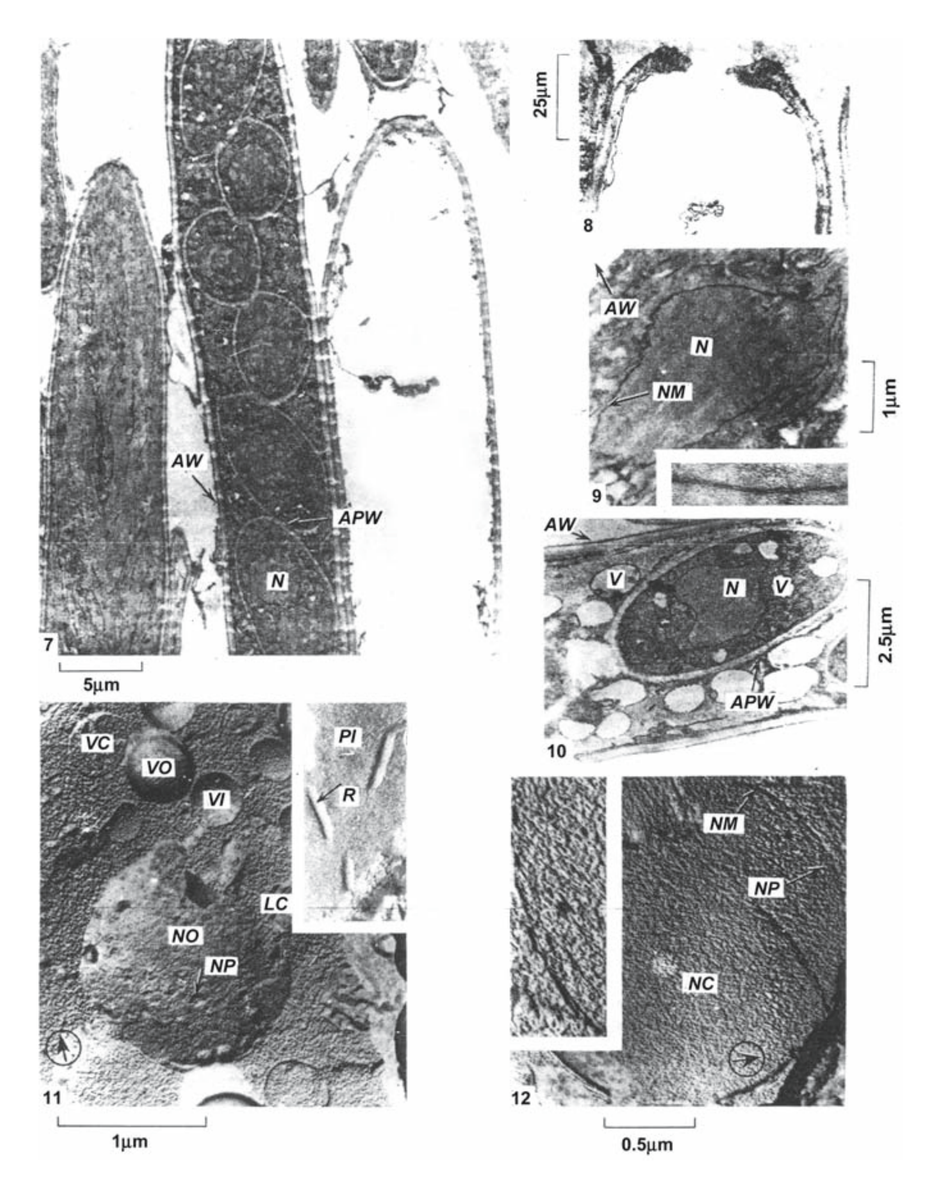


Plate 9.2.3.2 (7): L.S. apothecium showing mature asci with and without ascospores; (8) L.S. empty ascus illustrating aperture at tip; (9) L.S. immature ascus, ascospore wall has not yet formed. Inset: detail of nuclear membrane; (10) L.S. mature ascus; (11) Part of the ascospore with various organelles, inset; inner surface of plasmalemma with ridges; (12) Cross fractured nucleus in ascospore, Inset: detail of two layered nuclear membrane (Adapted from the publication of Jones, 1974a. With permission)

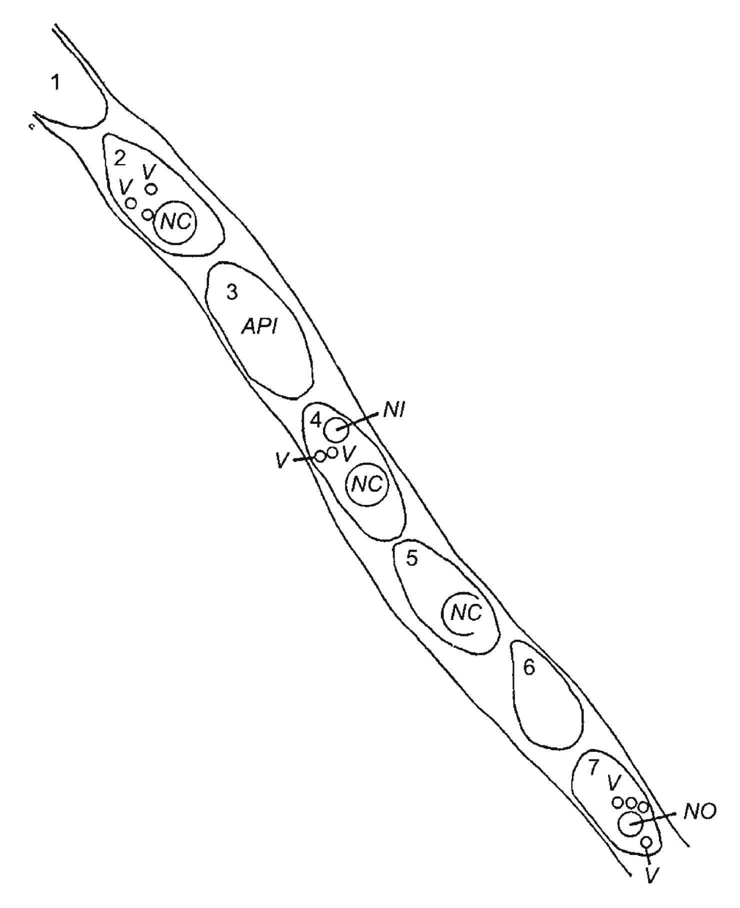


Plate 9.2.3.3 Semi diagrammatic drawing of a freeze fractured ascus containing ascospores (Adapted from the publication of Jones, 1974a. With permission)

9.2.4 Ultra-structures of Microconidia and Stroma

The microconidia shows the usual cell organelles already described for *S. sclerotiorum*. A large nucleus, large lipid body, a few mitochondria and sparse endoplasmic reticulum are present. The stromatal hyphae are with a rich content in lipid bodies and storage vacuoles as food reserve. Simple septa with plugged pores are occasionally seen. Two kinds of hyphae exist, one has abundant food material and the other has degenerated aspect. Both hyphae show a similar thickness in their cell walls approximately 0.2 µm with the 1.5–3 µm diameters (Calonge, 1970).

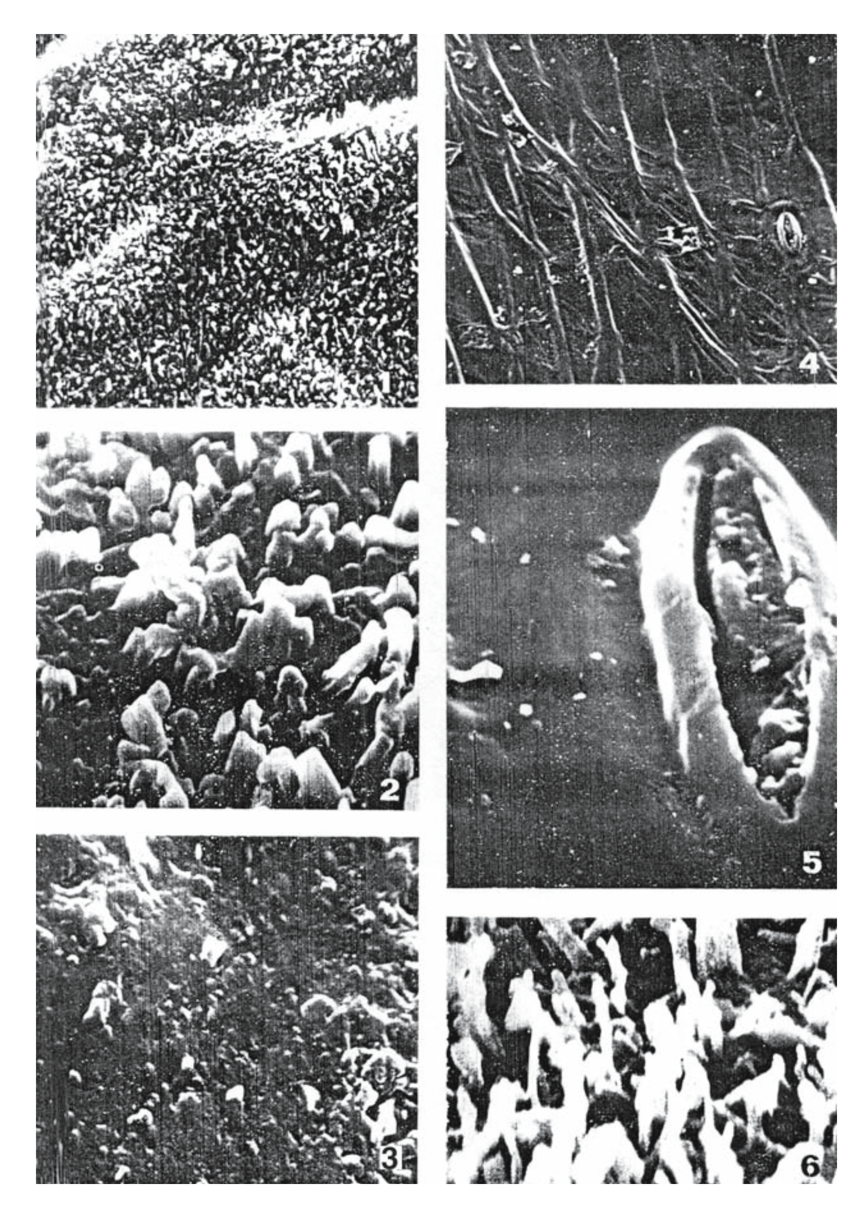


Plate 9.3.1 Scanning electron micrographs of stem and leaf surface of *Pisum sativum* infected with *Sclerotinia sclerotiorum*. (Figs. 1–2) Parchment like tissue of stem lesions showing profusion of wax like rodlets; (Fig. 3) Healthy green parts of stem surrounding white area showing few cuticular wax protuberances; (Figs. 4, 5) Stem lesions after immersion in petroleum ether. Note stomatal opening; (Fig. 6) Ribbon like cuticular wax at lower leaf surface (Adapted from the publication of Jones, 1976. With permission)

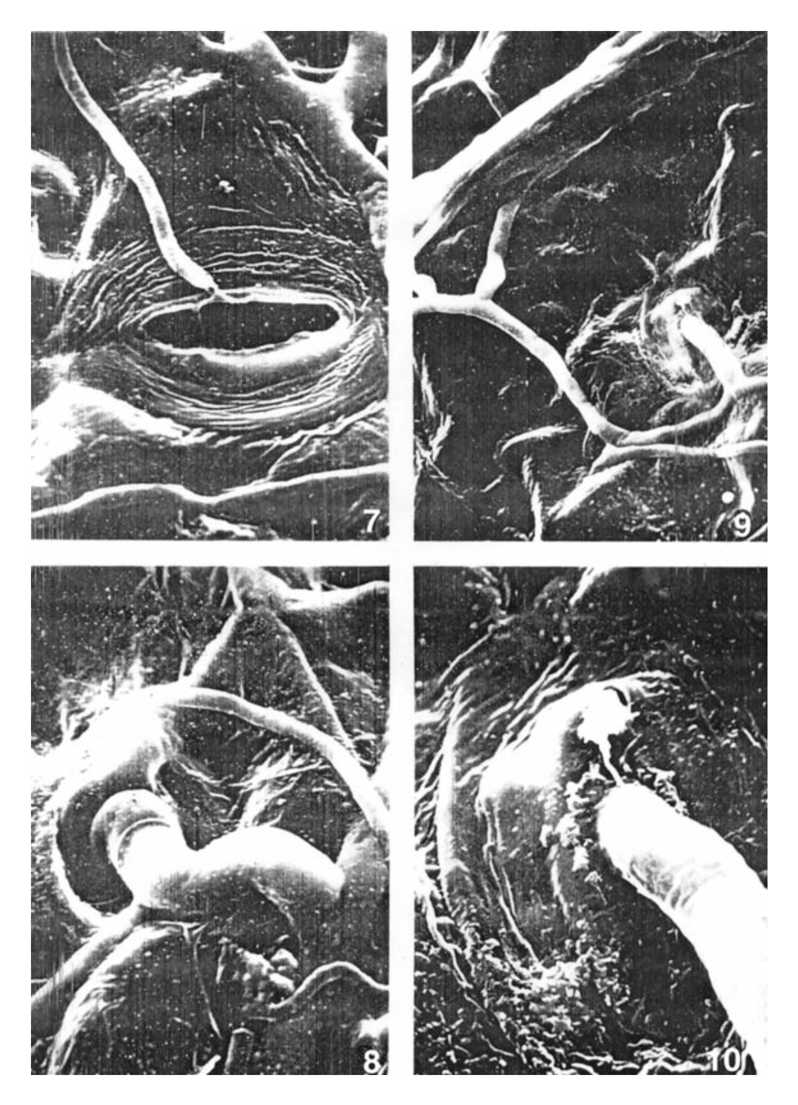


Plate 9.3.2 (Figs. 7–10). Scanning electron micrpgraphs of *Sclerotinia sclerotiorum* hyphae penetrating stomatal openings of the adaxial surface of a potato leaf (Adapted from the publication of Jones, 1976. With permission)

9.3 The Host-Pathogen Interface

The direct invasion of intact host surfaces by pathogenic fungi is common to many host-parasite interactions. On intact aerial surfaces of plants the cuticle constitutes the first barrier to infection and whether its penetration is achieved merely by the physical force exerted by an infection structure, or by enzymatic digestion has long

been debated (Kolattukudy, 1985). There are number of reports (Abawi et al., 1975a; Lumsden and Dow, 1973; Prior and Owen, 1964; Purdy, 1958; Tariq, 1984; Tariq and Jeffries, 1984, 1986) that in general, penetration of host tissues by *Sclerotinia* occurs directly through the cuticle, although entry via open stomata has been observed in some cases (Jones, 1976; Tariq, 1984). A scanning electron microscopic (Plates 9.3.1, 9.3.2) study employed by Jones (1976) revealed that *S. sclerotiorum* hyphae penetrate stomatal openings on the adaxial leaf surfaces of potato plants. Scanning electron microscopy of the infection process by *S. minor* and *S. sclerotiorum* corroborates the findings of light microscopy studies with *S. minor* (Lumsden and Dow, 1973), *S. sclerotiorum* (Abawi et al., 1975a; Boyle, 1921; Lumsden and Dow, 1973; Purdy, 1958) and *S. trifoliorum* (Prior and Owen, 1964). The infection process is characterized by the formation of infection cushions on the host surface, mechanical penetration of the cuticle, the formation of inflated infection hyphae that develop intercellularly, inter and intracellular colonization of the host tissue by ramifying hyphae, the emergence of hyphae through the stomata and the formation of sclerotia and dense mycelial mats on the host surface (Lumsden and Wergin, 1980).

Appressoria develop on the surface of the host prior to penetration, their formation being a pre-requisite to the invasion of healthy host tissue via cuticle. Appressoria adhere tightly to the host surface and appear to be cemented to the latter by means of a mucilaginous material (Boyle, 1921; Tariq, 1984; Tariq and Jeffries, 1984, 1986). Several workers (Abawi et al., 1975; Boyle, 1921; Lumsden and Dow, 1973, Tariq, 1984) have suggested that this material, in conjunction with the domed shape of the appressorium, allowed the appressorium to exert considerable force on the cuticle and thus mechanically gain entry into the underlying host tissue via the narrow infection pegs that form at the tips of appressorial hyphae. The theory that mechanical force alone is probably responsible for entry of the pathogen is supported by the following evidence: infection cushions (appressoria) often appear to pinch the surrounding host tissue into a slightly convex mound (Lumsden and Dow, 1973), the cuticle is pushed inward at points of penetration (Boyle, 1921; Lumsden and Dow, 1973). There is no softening, dissolution, or any modification of the cuticle prior to penetration (Boyle, 1921) and the cuticle remains impervious to the fungus (Lumsden and Dow, 1973) and shows no alteration in staining reaction even late in pathogenesis (Boyle, 1921; Lumsden and Dow, 1973). Prior and Owen (1964) while attributing the ability of *S. trifoliorum* to penetrate clover leaves to the mechanical force exerted by the infection cushions, reported disintegration of the cuticle and epidermal cells which apparently result from enzyme activity of the appressoria. Latterly there have been several reports of cutin degradation *in-vitro* by plant pathogenic fungi (Kolattukudy, 1985).

Histological studies at an ultra-structural level of the infection of plant tissues by fungal pathogens indicate that enzymes may be involved in host penetration, while histochemical studies at the light microscope level demonstrated the presence of esterase activity and chemical modification of cutin at the sites of penetration. Tariq and Jeffries (1986) demonstrated characteristic changes in chloroplast ultra-structure in *Phaseolus* during invasion by *Sclerotinia* (Tariq and Jeffries, 1985) and

indicated that cell wall degrading enzymes can be important in the penetration process (Tariq, 1984). Furthermore, Tariq and Jeffries (1987) are able to induce non-specific esterase activity in liquid cultures of *S. sclerotiorum* by adding cutin substrates. Lipolytic enzyme activity is localized during the penetration of *Phaseolus* leaf tissues by *S. sclerotiorum*. Enzyme activity is initially observed at sites where an appressorium appears in close contact with the host surface. Electron opaque deposits are formed in the region directly below a vesicular region and an extra-plasmalemmmal zone is present in the fungal cytoplasm. It appears to be restricted to the region between the plasma lemma of the fungus and the host cuticle. The electron opaque deposition is observed only at the site of host penetration in the later stages of infection in the vicinity of the penetration pore (Tariq and Jeffries, 1987).

Chapter 10
Pathogenic Variability

Considerable variation in morphology (Marukawa et al., 1975a; Morrall et al., 1972; Price and Calhoun, 1975a) and pathogenicity (Marciano et al., 1983; Morrall et al., 1972; Price and Calhoun, 1975a) has been observed among isolates of *S. sclerotiorum*. Variation in pathogenicity have been associated with the production of pectolytic enzymes (Hancock, 1966; Lumsden, 1976, 1979), cellulase (Lumsden, 1969), hemicellulase (Hancock, 1967), phosphatidase (Lumsden, 1970), and oxalic acid (Maxwell and Lumsden, 1970; Marciano et al., 1983; Noyes and Hancock, 1981). However, variation in these factors does not appear to be the primary determinant of pathogenicity as correlations have not always been apparent (Marciano et al., 1983; Morrall et al., 1972). In *S. trifoliorum*, a degenerate non-virulent isolate of the pathogen produces more protopectinase than a normal strain but only the normal isolate secretes a toxin and is considered virulent (Held, 1955). Little information is available on additional mechanisms that affect virulence among isolates of *S. sclerotiorum*.

Hypovirulence has been viewed as the reduced ability of a pathogen to infect, colonize, kill and reproduce in susceptible host tissue. Hypovirulence has been associated with genetic or cytoplasmic determinants such as nuclear and extranuclear genetic elements, heterokaryons, organelles, viruses, virus-like agents and plasmids (Elliston, 1982). Several fungal plant pathogens have been reported to contain mycoviruses, virus-like particles, or double-stranded ribonucleic acids (ds RNA) that are associated with reduced pathogenicity and/or reduced growth rate and reproductive fitness (Buck, 1986, 1987; Elliston, 1982; Ghabrial, 1980; Nuss and Koltin, 1990; Van Alfen, 1982). The detection of ds RNA in fungi is often considered to be indicative of the presence of mycoviruses, most of which contain ds RNA as genetic material (Lemke and Nash, 1974; Ralph, 1969). Recently, a slow growing isolate of *S. sclerotiorum* was recovered in culture that, in preliminary experiments was hypovirulent on bean and celery in comparison to typical, virulent isolates. The presence of mycoviruses, virus-like particles, or ds RNA in *Sclerotinia* spp. has not been reported but the widespread presence of these agents in fungi and their possible influence on virulence suggests that such parasites may contribute to variations in cultural morphology and virulence among isolates of *S. sclerotiorum* (Boland, 1987, 1988). Boland (1992) compared five isolates of *S. sclerotiorum* for morphological differences in agar culture, virulence on celery petioles, and the

G.S. Saharan, Naresh Mehta, *Sclerotinia Diseases of Crop Plants: Biology, Ecology and Disease Management*,

presence of double stranded ribonucleic acids (ds RNA). Four isolates grow rapidly in culture, develop typical colony morphologies are virulent on celery petioles and do not contain ds RNA. The fifth isolate (isolate 91) grows slowly in culture, develops typical colony morphology, produces significantly smaller lesions on celery than virulent isolates and contains ds RNA. Isolate 91 is considered to be hypovirulent. Hypovirulence is transmitted from isolate 91 to virulent, recipient isolates by hyphal anastomosis. Recipient isolates grow slowly in culture, develop a typical colony morphologies are hypovirulent and contain ds RNA. Initiating cultures from hyphal tips and treating colonies with heat or cycloheximide does not eliminate ds RNA from isolate 91. Hypo-virulence in isolate 91 of *S. sclerotiorum* is associated with the presence of ds RNA. The hypo-virulent phenotype of isolate 91 includes symptoms of both reduced virulence and debilitation. Hypovirulence is transmissible in *S. minor* (Melzer and Boland, 1996). The existence of dsRNA – free hypovirulence in S10 progenies, observed by Li-Guo Quing et al. (2003b) suggests that another hypovirulence mechanism may exist in *S. sclerotiorum*.

In a study (Kohn et al., 1990, 1991) conducted in Ontario Canada, each of two field populations of *S. sclerotiorum* on canola has been shown to be genetically heterogenous, i.e., composed of several clones, each clone capable of increasing through asexual or homothallic sexual reproduction. These clones are identified by two independent approaches, determination of mycelial compatability groups (MCGs) and assay of molecular markers. All three molecular markers demonstrating intraspecific variability shows most MCGs to be genetically uniform. One of the moleular markers is a dispersed repetitive DNA sequence, pLKUu 20, which when used as a cloned probe in southern hybridizations produces a unique, complex hybridization pattern, a DNA fingerprint, for each MCG. Monosporous isolates of field-collected apothecia show no evidence of recombination among the determinants of mycelial compatibility or DNA fingerprint. This homogeneity suggests that clones reproduce by inbred homothallic fruiting, in addition to asexual means. This study determines that mycelial compatibility and DNA fingerprinting with pLKUμ 20 are effective tools for identifying clones of *S. sclerotiorum*. Clonal variability within and among field populations of *S. sclerotiorum* isolates from canola petals in Western Canada has been determined (Kohli et al., 1992) by analysis of two independent criteria, mycelial compatibility and DNA fingerprinting. Strains are considered to belong to the same clone if they are mycelially compatible and also have identical DNA fingerprints. Thirty nine clones have been identified among 66 strains from seven locations in Alberta, Saskatchewan and Manitoba. The most widely distributed clone, accounting for 18 per cent of the isolates has been recorded in all three provinces. In 33 out of 36 mycelial compatibility groups (MCGs), each MCG has a unique DNA fingerprint; each of the remaining three MCGs includes strains with one of two fingerprints and is interpreted as two clones. A comparison of strains from Western Canada with those from a previous study of two fields in Ontario shows that the one clone identified in both Ontario fields is also present in Manitoba and Saskatchewan. This study demonstrates that clones of *S. sclerotiorum* are distributed over long distances geographically and confirms the results of the Ontario study in demonstrating that field populations of *S. sclerotiorum* on canola are

composed of more than one clone. Analysis of monosporous siblings from homothallic sexual reproduction in each of two clones shows no meiotic segregation for determinants of either mycelial compatibility or DNA fingerprints. Therefore, intact clonal genotypes can potentially be dispersed as ascospores.

According to Errampalli and Kohn (1996), Electrophoretic karyotypes (EKs) of 83 isolates are variable within agricultural and natural populations of *Sclerotinia sclerotiorum* as well as among *S. minor* and *S. trifoliorum*. Variation in EKs is not observed within six mitotic or three meiotic lineages of isolates. EKs of eight to ten chromosome-size DNAs are observed. Homologous and heterologous probes hybridize to four linkage groups.

Molecular techniques have been used to characterize different field isolates of *S. sclerotiorum*. Chromosome DNA resolves by pulsed field gel electrophoresis (PFGE) revealed that *S. sclerotiorum* contains at least 16 chromosomes ranging from 1.5–4.0 Mb. The size of the haploid genome is estimated to be 43.5 Mb. Six field isolates with different levels of virulence on sunflower germlings or green beans are differentiated by random amplification of polymorphic DNA (RAPD), and analyzed by clamped homogenous electric field electrophoresis. This analysis reveals few chromosome-length polymorphisms among these strains. Chromosomal DNA hybridization indicates that the endopolygalacturonase-encoding *pgl* gene is localized on the smallest chromosome of all the strains, whereas the ribosomal DNA mapped to different sized chromosomes. The less aggressive strain is characterized by the presence of a supernumerary small band, presumably consisting of ds RNA. In contrast to numerous other phytopathogenic fungi, this study reveals a strong karyotypic stability among the strains of *S. sclerotiorum* which may be preserved by the sexual mode of reproduction of this species (Fraissinet-Tachet et al., 1996).

Sclerotinia sclerotiorum produces several polygalacturonases which together with other pectinolytic enzymes are also involved in the degradation of pectin. A number of different genomic clones have been isolated by screening a genomic DNA library in phage EMBL 3. Southern-blot and restriction mapping indicates that seven genes constitute two subfamilies of a multigene family encoding endopolygalacturonase. Using pulsed field gel electrophoresis to separate *S. sclerotiorum* chromosomes each subfamily is found to hybridize to a different chromosome. A comparison of the nucleotide sequence for the coding region of three members of the gene family reveals surprisingly few base substitutions suggesting that this gene family arise from recent multiple duplication events (Fraissinet-Tachet et al., 1995).

Analysis of mitochondrial DNA (mtDNA) haplotypes of *S. sclerotiorum* points to a common origin of some genotypes from agricultural populations especially when compared with two wild populations which are sharply distinguished from the agricultural sample and from each other. Five agricultural population samples from canola (Alberta, Canada and Norway), cabbage (North Carolina, USA), sunflower (Manitoba, Canada and Queensland, Australia) and two Norweigan populations from a wild plant, *Ranunculus ficaria* have been compared. Haplotypes are determined by southern hybridization of purified organelle DNA from *S. sclerotiorum* and *Neurospora crassa* to total genomic DNA of *S. sclerotiorum*. Each isolate has one

haplotype. Haplotypes of *S. sclerotiorum* from *R. ficaria* are different between the two wild populations and also from all haplotypes observed in the agricultural populations. Among the wild isolates, DNA fingerprint, mtDNA haplotype and location in the sampling transect are all associated. Among the agricultural isolates, four haplotypes have been observed in at least two agricultural populations and one haplotype has been observed in all agricultural populations. In the Canadian canola sample, some clones have one mtDNA haplotype, indicating association with DNA fingerprint, some clones have more than one haplotype and some groups of clones share haplotypes. Some of the haplotype diversity may be due to the presence of extra-chromosomal elements associated with the mitochondia of *S. sclerotiorum* (Kohli and Kohn, 1996). Eighty four isolates of *S. sclerotiorum* from four cabbage production fields in North Carolina and 16 isolates from an experimental cabbage field plot in Louisiana were DNA fingerprinted and tested for mycelial compatibility by Cubeta et al. (1997). In a comparison with 594 unique DNA fingerprints of *S. sclerotiorum* from Canadian canola, no fingerprints are shared among Canadian, North Carolina and Louisiana populations. DNA fingerprints from the North Carolina sample are distinctive from those of the Canadian and Louisiana samples with significantly more hybridizing fragments in the 7.7–18 kb range. Forty-one mycelial compatibility groups (MCGs) and 50 unique DNA fingerprints are identified from the North Carolina sample. Three MCGs and three fingerprints are identified from the Louisiana sample. From the North Carolina sample, 32 MCGs are each associated with a unique fingerprint and results revealed that there are 11 clones (i.e., cases in which two or more isolates belong to the same MCG and share the same DNA fingerprint). Six clones sampled from two or more fields represent approximately 29 per cent of the total sample (24 of 84 isolates) with six clones recovered from fields 75 km apart. There are ten cases in which one MCG is associated with more than one DNA fingerprint and two cases in which one DNA fingerprint is associated with more than one MCG. The small sample from Louisiana is strictly clonal. The North Carolina sample has a clonal component, but deviates from one to one, association of MCG with DNA fingerprint to an extent consistent with more recombination or transposition than the other two populations sampled.

Population variability of *S. sclerotiorum* causing stem rot of soybean has been determined by mycelial compatibility grouping (MCG) and isolate aggressiveness comparisons (Kull et al., 2004). Within widely dispersed MCGs, isolate aggressiveness varies. Mycelial compatibility groups of 47 strains of *S. sclerotiorum* have been investigated from Hungary by Zandoki et al. (2005a) along with great number of vegetative compatibility groups.

According to Maltby and Mihail (1997), populations of *Sclerotinia sclerotiorum* are often composed of multiple genotypes. In examining 35 naturally infected canola plants, 29 supported reproduction (i.e. sclerotium formation) by a single *S. sclerotiorum* genotype, as defined by the mycelial compatibility test. Only six plants supported reproduction by two genotypes. To test the hypothesis that infrequent multiple genotype infections are due to differences in virulence or competitive ability among isolates, four greenhouse experiments have been conducted in which

four isolates, representing three genotypes are used in pair wise co-inoculations of canola. There are no differences among the isolates in four virulence parameters. Mean reduction in sclerotial mass produced by a co-inoculated isolate is calculated by comparison with the mean sclerotial mass of that isolate in the absence of competition and used as the measure of competition. In all experiments, at least half of the co inoculation treatments resulted in reduced fungal reproduction for one or both of the co-inoculated isolates, providing evidence of competitive differences. Generally, the magnitude of reproductive reduction is the same for each isolate in the pair. However, the magnitude is nonreciprocal when the more competitive isolate is given an advantage of early temporal arrival or spatial placement of inoculum at the lower position on the stem. Competitive differences among *S. sclerotiorum* isolates affecting reproduction represent one possible mechanism to explain temporal shifts in genotypic frequencies.

Ghasolia and Shivpuri (2007) studied the morphological and pathogenic variability in 38 isolates of *Sclerotinia sclerotiorum* collected from different rapeseed-mustard fields in Rajasthan State (India). Studies revealed that there are nine groups of the isolates and among these, group G_6 was the most virulent causing maximum disease incidence (82.44 per cent) followed by G_1 (77.50 per cent) and G_8 (75.01 per cent) while G_9 had minimum disease incidence (11.08 per cent). Based on the degree of aggressiveness, nine groups were devided into two pathotypes i.e., pathotypes-I (G_2, G_5, G_9 as these caused less than 18 per cent disease incidence and pathotypes II includes G_1, G_3,G_4, G_6, G_7 and G_8 caused more than 66 per cent disease incidence.

According to Nedeleu et al. (1988) *Sclerotinia* isolates with small sclerotia (0.3–0.5 cm) are more virulent than those with large sclerotia (1.0–1.5 cm). Isolates from the weeds are more pathogenic than those from soybean.

10.1 Genetic Analysis of Isolates

Heterokaryon formation and vegetative compatibility in *S. sclerotiorum* has been observed by Ford et al. (1995). When auxotrophic mutants derived from six prototrophic wild types are paired in combinations then some combinations of auxotrophs lead to the development of prototrophic strains, the prototrophic strains and heterokaryons. Heterokaryotic formation is under the control of a regulatory system that results in incompatibility in some strains. Vegetative compatibility groups do not directly correspond to mycelial compatibility groups. Telomere sequence based for revealing genotypic differences among isolates of *S. sclerotiorum* have been suggested (Meinhardt et al., 2002).

According to Steadman et al. (1998), Random Amplified Polymorphic DNA (RAPD) distinguishes three species of *Sclerotinia* but not pathogenic variability in *S. sclerotiorum* isolates from diverse host and geographic origin. Virulence as a trait may be environmentally plastic. Isolates from the same host differs in virulence.

The genetic diversity and genetic structure of a population of isolates of *Sclerotinia sclerotiorum* (Lib.) de Bary from different regions and host plants have been investigated using the random amplified polymorphic DNA (RAPD) method with 20 random decamer primer pairs in order to provide some information on the phylogenetic taxa and breeding for resistance to *Sclerotinia* stem rot. A minimum of three and a maximum of 15 unambiguously amplified bands are generated, furnishing a total of 170 bands ranging in size from 100–3,200 bp, corresponding to an average of 8.5 bands per primer pair. One hundred and four of these 170 bands (61.2 per cent) are polymorphic, the percentage of polymorphic bands for each primer pair ranging from 0.0 to 86.7 per cent. The genetic relationships among the isolates, based on the results of RAPD analysis are examined. The genetic similarity of all selected isolates is quite high. At the species level, the genetic diversity estimated by Nei's gene diversity (h) is 0.197 and Shannon's index of diversity (I) is 0.300. The unweighted pair-group mean analysis (UPGMA) cluster analysis shows that most isolates from the same regions are grouped in the same cluster or a close cluster. The population of isolates from Hefei (Anhui Province, China) is more uniform and relatively distant to other populations. The Canadian population collected from carrot (*Daucus carota* var. *sativa* DC.) is relatively close to the Polish population collected from oilseed rape (*Brassica napus* L.) plants. There is no relationship between isolates from the same host plants. An analysis of molecular variance (AMOVA) revealed that the percentage of variance attributable to variation among and within populations is 50.62 and 49.38 per cent respectively. When accessions from China, Europe, and Canada are treated as three separate groups, the variance components among groups, among populations within groups, and within populations are 0.96, 51.48 and 49.47 per cent respectively. The genetic differentiations among and within populations are highly significant. Similarly, the coefficient of gene differentiation (Gst) in total populations calculated by population genetic analysis is 0.2294, which indicates that the genetic variation among populations is 22.94 per cent. The gene flow (Nm) is 1.68, which indicates that the gene permutation and interaction among populations is relatively high (Sun-Jun Ming et al., 2005). According to Noonan et al. (1996), isolates of *S. minor* and *S. trifoliorum* are clearly separate from *S. sclerotiorum* using RAPD, but no differences are detected in r DNA. Genetic diversity exists in the isolates of *S. sclerotiorum* from rapeseed, soybean and sunflower but there is no correlation between the genetic diversities and virulence differentiation (Li-Yong Hong et al., 2005).

10.2 Population Biology

To achieve genetic diversity in *Sclerotinia* populations worldwide, we should use the same genetic markers, expand and cross reference data bases, use the same positive controls standards and voucher isolates and provides genotyped and tested isolates of epidemiological significance for research on pathogenesis, control and resistance (Kohn, 2001).

Population studies on *S. sclerotiorum* have revealed a predominantly clonal mode of reproduction (Cubeta et al., 1997; Kohli and Kohn, 1998; Kohli et al., 1995) with some evidence of out crossing contributing to the population structure in a few regions (Atallah et al., 2004; Kohli and Kohn, 1998; Sexton and Howlett, 2004). In Australia and some temperate regions of North America, populations of *S. sclerotiorum* show some genetic diversity through out crossing in addition to clonal reproduction (Atallah et al., 2004; Sexton and Howlett, 2004) while in Canadian oilseed rape fields, there is no evidence of sexual recombination within a large population composed of a small number of clones, with a single clone repeatedly isolated across 2,000 km over a four year period (Anderson and Kohn, 1995; Kohli et al., 1992). *S. sclerotiorum* has a haploid somatic phase where clonality is the result of both asexual reproduction by means of sclerotia and sexual reproduction by self fertilization (Kohn, 1995) with the expectation that intraclonal variation is due to mutation (Carbone and Kohn, 2001; Carbone et al., 1999).

Individual isolates are classified into clonal lineages by the use of two or more independent markers such as mycelial compatibility groups (MCGs), DNA fingerprinting or microsatellites (Auclair et al., 2004a; Carbone et al., 1999; Hambleton et al., 2002; Kohn et al., 1991; Sirjusingh and Kohn, 2001). MCG testing is a phenotypic, macroscopic assay of the self/non self recognition system controlled by multiple loci common in fungi (Carbone et al., 1999). Mycelial incompatibility is a failure of different strains to fuse and form one cohesive colony and is characterized by the formation of dead cells and reduced growth between the two incompatible colonies (Kohn et al., 1991). DNA fingerprinting utilizes a probe for a multicopy transposon like element in Southern analyses (Kohn et al., 1991). Microsatellite loci have high mutation rates and are multiallelic in nature, making them useful in phylogenetic inference (Sirjusingh and Kohn, 2001). In general, these studies have shown an association between MCGs, DNA fingerprints and microsatellite markers (Carpenter et al., 1999; Hambleton et al., 2002; Sexton and Howlett, 2004), but Atallah et al. (2004) recently reported no association between MCGs and microsatellite markers with isolates from potato.

MCGs or microsatellite markers have not been associated with specific virulence characteristics or ecological adaptations of the pathogen indeed, a lack of variation in virulence among isolates from defined geographical areas has been reported in a number of studies on agricultural populations (Atallah et al., 2004; Auclair et al., 2004a; Kull et al., 2004; Sexton and Howlett, 2004). Differences in virulence may be detected when comparing isolates form widely separate geographical regions. There has been no conclusive evidence to suggest host specialization among isolates of *S. sclerotiorum* (Kull et al., 2004). However, comparison of *S. sclerotiorum* populations on cultivated oilseed rape and on the wild perennial host *Ranunculus ficaria* indicated major differences between agricultural and wild populations (Kohn, 1988). DNA fingerprint diversity is high in agricultural populations but low in wild populations and there is no evidence of out crossing in agricultural populations even though recombination occurs in wild populations.

10.3 *Agrobacterium*-Mediated Transformation of *Sclerotinia sclerotiorum*

Ascospores from the phytopathogenic fungus *Sclerotinia sclerotiorum* are transformed to hygromycin B resistance by co-cultivation with *Agrobacterium tumefaciens*. Transformed spores germinate and grow on PDA supplemented with 100 μg/ml hygromycin B. The presence of mitotically stable *hph* gene integration at random sites in the genome is confirmed by PCR and Southern blot analysis. A transformation frequency of 8×10^{-5} is achieved. A reproducible *Agrobacterium*-mediated transformation method should allow the development of T-DNA tagging as a system for insertional mutagenesis in *S. sclerotiorum* and provide a simple and reliable method for genetic manipulation (Weld et al., 2006).

10.4 A Group-I Intron in the Mitochondrial Small Subunit Ribosomal RNA Gene of *Sclerotinia*

A 1,380-bp intervening sequence within the mitochondrial small subunit ribosomal RNA (mt SSU rRNA) gene of *S. sclerotiorum* has been sequenced and identified as a group-I intron. This is the first report of an intron in the mt SSU rRNA gene. The intron shows close similarity in secondary structure to the subgroup-IC2 introns from *Podospora* (ND3i1, ND5i2, and COIi5) and *Neurospora* (ND5i1). The intron has an open reading frame (ORF) that encodes a putative protein of 420 amino acids which contains two copies of the LAGLI-DADG motif. The ORF belongs to a family of ORFs identified in *Podospora* (ND3i1, ND4Li1, ND4Li2, ND5i2, and COIi5) and *Neurospora* (ND5i1). The putative 420-aa polypeptide is also similar to a site-specific endonuclease in the chloroplast large subunit ribosomal RNA (LSU rRNA) gene of the green alga *Chlamydomonas eugametos*. In each clone of *S. sclerotiorum* examined, including several clones which were sampled over a three-year period from geographically separated sites, all isolates either has the intron or lacked the intron within the mt SSU rRNA gene. Screening by means of Southern hybridization and PCR amplification detects the intron in the mt SSU rRNA genes of *S. minor*, *S. trifoliorum* and *Sclerotium cepivorum*, but not in other members of the Sclerotiniaceae, such as *Botrytis* anamorphs of *Botryotinia* spp., or in other ascomycetous and basidiomycetous fungi (Carbone et al., 1995).

Chapter 11
Perpetuation

The pathogen over winters as mycelium in dead or living plants and as sclerotia on or within infected tissues or as sclerotia that have fallen on the ground or through infected seed and propagating materials. Being a wide host pathogen various hosts provide good opportunity for successful survival of the pathogen under all possible adverse conditions. Under favourable environmental conditions, the sclerotia germinate and produce one to many slender stalks terminating at a small, 5–15 mm in diameter, disc or cup-shaped apothecium in which asci and ascospores are produced. Large numbers of ascospores are discharged from the apothecia into the air over a period of two to three weeks. The ascospores are blown away and if land on susceptible plant parts, germinate and cause infection. Very frequently, the sclerotia cause infection by producing mycelial strands, which attack and infect young plant stems directly. Under moist conditions, the latter method of infection is probably more common than the one by ascospores (Abawi and Grogan, 1975; Agrios, 2005; Sherf and Macnab, 1986; Walker, 1969). According to Tu (1988), *Sclerotinia sclerotiorum* survives in infected seeds of white bean as dormant mycelium in testa and cotyledons over a three year period up to 85–89 per cent. Under favourable conditions, sclerotia formed on infected seeds may be capable of producing apothecia later in the same season (Hungerford and Pitts, 1953).

The sclerotia produced by *S. sclerotiorum* in sunflower plants measure in the range of 11–18 mm in length (Young and Morris, 1927). Formation of secondary sclerotia, which insures the persistence of inoculum in soil even in the absence of sunflower or other host species is also reported (Huang, 1981). Hoes and Huang (1975) reported ten times more occurrence of sclerotia in the rhizosphere soil (12 sclerotia/500 g of soil) from sunflower with root rot than in controls. The heavily infested soil under Taiwan conditions has been found to have 22–40 apothecia/m^2 (emerged through sclerotial germination) during the sunflower growing period (Wu, 1981). Besides its survival in soil, the seeds of the crop also get contaminated mechanically with sclerotial bodies which are of the same size and colour as sunflower seed. Mycelial strands have been observed in thick-walled fibrous cells of the pericarps as well as in the thin-walled parenchyma cells of the testa and the inner layer of the pericarp (Tollenaar and Beleiholder, 1972). Wilt incidence is reported to be 95 per cent in a field sown with seed contaminated with 1 per cent sclerotia under favourable environmental conditions (Hoes and Huang, 1976). In a partially affected

G.S. Saharan, Naresh Mehta, *Sclerotinia Diseases of Crop Plants: Biology, Ecology and Disease Management*,

flower head, presence of sclerotia on a well-developed seed surface may also become visible. In this way, the seed is likely to become a source of inoculum and agent of dissemination of the pathogen from one area to another (Kushal and Saharan, 1999).

The sclerotia have been found to retain viability and virulence for seven years (Spitsyn and Kochenkova, 1978). However, viability of the sclerotia depends on the type of sclerotium itself and several environmental factors. Small sclerotia have less reserve food, and these are more easily destroyed by soil organisms than large sclerotia. It is reported that moist sclerotia die rapidly, whereas the dry ones remain viable at 3°C for 480 days and at 8°C up to 300 days (Shopov, 1976). According to Nisikado and Hirata (1937), sclerotia do not survive more than two years or more at 20°C or over 14 months at 25°C, 10 to 14 months at 30°C and 3 to 4 months at 35°C. The newly formed sclerotia do not germinate. Viability of sclerotia decreases with degree of abnormality (Huang and Kozub, 1994). However, according to Quentin (2004), sclerotia of *S. sclerotiorum* can survive for up to ten years in the soil, benefiting from irrigation and warmer climates.

Mycelium of the pathogen grows saprophytically (Bisby, 1924) and over winters on sunflower stalks (Young and Morris, 1927). Residues of the crop thus may provide host tissue for development of mycelium in a situation where moisture and temperature are favourable. The mycelium has been reported to remain infective at −8°C and it is interesting that the desiccated powdered mycelium has been reported to preserve its infectivity for a considerable period of time under the conditions of the Soviet Union (Nisikado and Hirata, 1937).

The pathogen is reported to survive in the form of ascospores to some extent if favourable temperature and RH is present under field as well as greenhouse conditions. Dry ascospores survive for a longer period of time. Therefore ascospores are helpful to act as a source of inoculum in some specific situations (Grogan and Abawi, 1975; Newton and Sequeira, 1972; Partyka and Mai, 1962; Caesar and Pearson, 1983).

11.1 Biology of *Sclerotinia*

During the life cycle *S. sclerotiorum* progress through three stages of development that include dormancy, saprophytism and parasitism. From a management perspective during sclerotial dormancy the pathogen is least accessible to most control methods. In contrast, the pathogen is more vulnerable during mycelial and carpogenic germination because of poor competitive saprophytic ability and high dependence on environmental factors, respectively. However, there are two key adaptation strategies during the saprophytic stage that contribute to the success of *S. sclerotiorum* in establishing a parasitic relationship with crops. First is the ability of the fungus to continue producing sclerotia on diseased foliar debris after being detached from the living plant, thus increasing the amount of inoculum in soil. Second is its ability to attune to crop phenology i.e., to develop apothecia and ascospores in synchrony with

the susceptible stage of crop. The pathogen may become less vulnerable to environmental stress or control methods during parasitism, when it is established in plant. Adverse conditions occurring during these growth stages may affect the overall development of the fungus. Therefore, knowledge of variations in the biological characteristics of *S. sclerotiorum* in relation to crop development and the physical environment within crops is important in designing management practices that aim at interrupting the life cycle of the pathogen.

11.1.1 Dormancy

Sclerotinia sclerotiorum spend about 90 per cent of its life cycle in soil as dormant sclerotia which develop primarily from mycelia on diseased tissues (Adams and Ayers, 1979). Mature sclerotia can survive in soil for one to five years depending upon the interaction of various physical and biological factors (Adams and Ayers, 1979; Cook et al., 1975). Physical factors in the environment, including prolonged period of high temperatures, flooding, sequential drying and wetting (Smith, 1972). Deep burial below the soil surface and exposure to solar radiation can reduce the viability of sclerotia (Adams and Ayers, 1979). However, microbial degradation remains the most significant factor affecting populations of sclerotia in natural ecosystem (Adams and Ayers, 1979). More than 100 species of fungi and bacteria (Table 19.9.1) have been identified as antagonists or mycoparasites of *Sclerotinia* species. But *Coniothyrium minitans*, *Trichoderma* spp. and *Sporidesmium sclerotivorum* are likely responsible for the destruction of the majority of sclerotia in soil (Adams and Ayers, 1979). Structural malformations or fractures in the melanized rind may also contribute to reduced longevity of sclerotia by increasing susceptibility to microbial degradation (Coley-Smith and Cooke, 1971). In addition soil mycophagus animals such as fungus gnats (*Bradysia coprophila*; Diptera: Sciaridae) and Springtails (*Folsomia candida*; Collembola; Entomobryidae) can reduce populations by direct consumption of sclerotia, mycoparasite transmission or predisposing damaged sclerotia to infection by mycoparasites (Anas and Reeleder, 1987; Gracia-Garza et al., 1997b; Godoy et al., 1990).

11.1.2 Saprophytism

Germination: During suitable environmental conditions, mature sclerotia can germinate myceliogenically to form mycelium or carpogenically to form apothecia (Adams and Ayers, 1979). During myceliogenic germination, sclerotia produce masses of mycelia which are capable of direct penetration of the host cuticle but require an exogenous nutrient source to be infective (Abawi and Grogan, 1975; Lumsden, 1979). Mycelia originating from sclerotia of *S. sclerotiorum* possess limited competitive saprophytic ability and in non-sterile soil are unable to infect

plants located more than 2 cm from the source (Newton and Sequeira, 1972; Williams and Western, 1965a).

Carpogenic germination requires functionally mature and preconditions sclerotia located in the upper 2–3 cm layer of soil (Abawi and Grogan, 1979). The dormancy period required before sclerotia can germinate carpogenically varies from 13–208 days depending on environmental and physiological factors (Willetts and Wong, 1980). Conditioning requirements for carpogenic germination origin of isolates as this relates to the temperature at which sclerotia are formed (Huang and Kozub, 1991b). Typically constitutive dormancy can be relieved by conditioning sclerotia for prolonged periods in cool, moist conditions or by over wintering in soil (Abawi and Grogan, 1979). Carpogenic germination of sclerotia occurs at soil matric potentials ranging from 0 to −7.5 bars (Boland and Hall, 1987; Morrall, 1977; Teo and Morrall, 1985a). However, moisture levels −0.1 to −0.4 bars are most favourable for apothecial production (Teo and Morrall, 1985a) and saturated soils (0 bars) may suppress germination due to lack of aeration or rotting of sclerotia (Morrall, 1977). Sclerotia can imbibe moisture up to amounts equivalent to their weight (Couper, 2001). In the laboratory, full hydration of sclerotia occurs within 4 h of immersion in water. While release of moisture from fully hydrated to fully desiccated sclerotia requires up to 25 h. Moisture content of soil buried sclerotia follows changes in the matric potential of the soil and can reach equilibrium within 6 h but sclerotia can remain partially hydrated in extremely dry soil (Couper, 2001). Typically, in the field conditions, development of apothecia is stimulated by extended periods e.g., ten days of high soil moisture potentials (0 to −0.3 bars), cool (4–20°C), soil temperature and a dense plant canopy over the soil (Abawi and Grogan, 1979; Grogan and Abawi, 1975; Morrall and Dueck, 1982; Schwartz and Steadman, 1978).

Carpogenic germination leading to formation of phototropic stipes and development of mature apothecia is optimal in soil temperature of 11–15°C. However, differentiation and full expansion of the apothecial disk and ascospores production occur only in light and require wavelengths below 390 nm and temperatures 15–20°C (Coley-Smith and Cooke, 1971; LeTourneau, 1979; Willetts and Wong, 1980). Mature ascospores are forcibly discharged to a distance of more than 1 cm above the upper surface of the apothecium when subjected to a decrease in moisture tension in the surrounding air (Abawi and Grogan, 1979). Apothecia can remain functional for five to ten days in field conditions and can produce up to 3×10^7 ascospores (Schwartz and Steadman, 1978; Steadman, 1983).

11.1.3 Aerobiology

Aerobiology studies of *S. sclerotiorum* in crops have revealed a diurnal distribution of ascospores characterized by consistent peaks of spore deposition occurring between 900 to 1,300 h in several geographical regions (Ben-Yephet and Bitton, 1985; Hartill, 1980; Hudyncia et al., 2000). Conversely seasonal patterns of

ascospores distribution vary among geographic regions because of differences in climates and cropping seasons. Peaks of ascospores deposition have been recorded during January and February in Israel (Ben-Yephet and Bitton, 1985), March in New Zealand (Hartill, 1980), July and August in Canada (Williams and Stelfox, 1980b) or October and November in North Carolina, USA (Hudyncia et al., 2000). Daily peaks in abundance of ascospores occur after sunrise, perhaps in response to increased light intensity and decreased RH, whereas seasonal peaks follow periods of high soil moisture (Hartill, 1980). Aerial dispersal of ascospores can reach up to several kilometers (Abawi and Grogan, 1979) but most of the ascospores are deposited within 100 (Ben-Yephet and Bitton, 1985; Steadman, 1983) or 150 m (Stelfox et al., 1978) from the source. Ungerminated ascospores can survive for up to 12 days in the crop depending on their position in the canopy and environmental conditions. Ascospores mortality increases with increasing temperatures above 21°C and exposure to ultraviolet radiation.

11.1.4 Adaptation

Ascospores of *S. sclerotiorum* are not able to infect until an external source of nutrition such as senescing plant tissues or dead blossoms and free water are available (Lumsden, 1979). However, unlike initial petal infestation in flowering plants such as bean (Abawi and Grogan, 1979) and canola (Mc Lean, 1958b), carrots, the exogenous food base for germinating ascospores is restricted to aged foliage. Carrot plants are not susceptible to foliage applied ascospores of *S. sclerotiorum* unless older senescing leaf tissues are present in canopy (Geary, 1978). Increased susceptibility of senescing leaves is associated with higher incidence of appressoria formation and failure to react hyper-sensitively to the penetration of *S. sclerotiorum*. In the field carrot crops are particularly susceptible to *S. sclerotiorum* when senescing foliage is lodged on the soil surface (Geary, 1978). This probably occurs because upright senescing leaves are subjected to continuous fluctuations and interruptions of leaf wetness periods due to wind and sunlight. Lodged senescing leaves can have longer wetness periods due to their contact with moist soil and humid conditions under the dense canopy cover, thus providing more favourable conditions for infection to occur. Therefore, the onset of lodged senescing leaves appears to be an important event in determining the susceptible stage of crops to infection by *S. sclerotiorum*. Once started foliar senescence of crops grasses concurrently with the development of new leaves and accumulation of senescing foliage on soil continues until harvest.

The pattern of foliage senescence differential susceptibility of leaves in relation to their age and position of older leaves within the canopy appears to be important for the development of *Sclerotinia* disease. Pattern of foliar senescence may vary with carrot age, cultivar, plant density, nutrient and irrigation regime, soil type or weather. In addition, stresses imposed by other foliage disease of carrots such as leaf blight (*Alternaria dauci* and *Cercospora carotae*) may encourage senescence and lodging.

The presence of apothecia and ascospores during this susceptible stage of carrot development has been observed (Couper, 2001; Geary, 1978). It is probably the most critical factor in the initiation of *Sclerotinia* rot of carrot epidemic. Emergence of apothecia has been associated with the development of a full canopy cover in carrot (Couper, 2001), and several other crops (Boland and Hall, 1987; Boland and Hall, 1988a; Morrall and Dueck, 1982; Schwartz and Steadman, 1978). An enclosed canopy can differ the effect of fluctuating soil moisture and temperature at the soil plant interface and create conditions that are conducive for the development of apothecia and survival of ascospores (Abawi and Grogan, 1979; Caesar and Pearson, 1983; Schwartz and Steadman, 1978; Weiss et al., 1980). The appearance of lodged, senescing leaves in carrot crops usually occurs close to or after full canopy enclosure. Therefore extended periods of concurrent production of inoculum and accumulation of susceptible tissues can cause wide spread epidemics of *Sclerotinia* rot of carrot particularly when favourable weather conditions prevail.

11.1.5 Parasitism

It has been discussed in detail in Chapter 12 under the head of infection and pathogenesis.

Chapter 12
Infection and Pathogenesis

The present knowledge about the process of infection and pathogenesis in the *Sclerotinia* disease is not complete and details about specific areas of physiology, biochemistry and molecular aspects especially are limited. However, in general, a clear understanding of the host-parasite interaction at the tissue level is available (Lumsden, 1979).

12.1 Penetration of the Host

The ability of *Sclerotinia* spp. to invade and the mode of penetration of host tissues depend upon the type of inoculum, the nutrient status of the fungus, the properties of the host and the effects of the surrounding environment. Two types of inoculum, germinated ascospores and mycelium from sclerotia or ascospores, can initiate infection. In some *Sclerotinia* caused diseases, ascospores are considered to be the primary source of inoculum. Germinated ascospores can produce a simple, single appressorium capable of entering the host if nutrients are present (Abawi et al., 1975 a & b; Purdy, 1958). Ascospores require external nutrients for penetration of the host. de Bary (1886, 1887) showed that *Sclerotinia* hyphae attack only after being properly "nourished and developed." Penetration occurs when hyphae are placed in a drop of nutrient solution on the host. In water alone, ascospores germinate but the hyphae are unable to form appressoria on the host surface and penetrate. Ascospores of *S. trifoliorum* from clover infect leaves of lettuce, broccoli, brussels sprouts, ladino clover, red clover, broad bean and snap bean (Purdy, 1958). In contrast, ascospores from lettuce and tomato isolates germinate but do not infect the host leaves unless they are partially senescent. Ascospores of all isolates require nutrients for infection to occur. Additionally, infection is usually directly through the cuticle, although germinating ascospores produce a diffusable substance that enters the stomatal opening and disorganizes the cellular contents. There are reports of entry of germ tubes via open stomata by mycelium of *S. trifoliorum* on a specific clover cultivar (Prior and Owen, 1964) and *S. sclerotiorum* on potato leaves (Jones, 1976). Penetration of most hosts, however, is not via stomata but directly through the cuticle (Abawi et al., 1975; Boyle, 1921; Lumsden and Dow, 1973;

G.S. Saharan, Naresh Mehta, *Sclerotinia Diseases of Crop Plants: Biology, Ecology and Disease Management*,

Prior and Owen, 1964; Purdy, 1958). Freeze and bruise injuries are important factors associated with infection of cabbage by *S. sclerotiorum* (Hudyncia et al., 2000).

Mycelial infection, rather than infection directly from germinated ascospores, appears to be the primary means of host penetration (Adams and Tate, 1976; Purdy, 1958). In addition, a source of organic matter for inoculum nutrition usually is a prerequisite for penetration, whether the original source of inoculum is germinated ascospores that invade senescent bean blossoms before infecting bean leaves (Abawi et al., 1975b) or germinated sclerotia (Purdy, 1958). Adams and Tate (1976) described an exception to that direct infection of lettuce plants by *S. minor* occurs in the absence of available organic matter. This exception may be due to the fact that sclerotia of *S. minor* can germinate by producing a mass or "plug" of mycelium that apparently has sufficient nutrient reserves to allow direct penetration. Sclerotia of *S. sclerotiorum* isolates have not been observed to germinate in this manner, but only by production of hyphal strands that require external organic matter before infection can occur (Purdy, 1958).

Appressoria are formed unless penetration occurs directly via stomata as with some hosts (Jones, 1976; Prior and Owen, 1964).Usually appressoria are complex, multicelled, dome-shaped structures variously referred to as appressorial masses (Purdy, 1958), large appressoria (Boyle, 1921), cushion-shaped appressoria (Abawi et al., 1975a) or infection cushions (Lumsden and Dow, 1973; Prior and Owen, 1964). Formation of these organized structures requires contact stimulus (Abawi et al., 1975a; de Bary, 1886; Purdy, 1958). After contact with the host, the hyphal strands branch dichotomously, form finger-shaped structures and eventually develop into dome-shaped infection cushions (Abawi et al., 1975a; Boyle, 1921; Lumsden and Dow, 1973; Purdy, 1958). Three distinct types of hyphae are observed in cross-sections of infection cushions (Lumsden and Dow, 1973), densely safranin-staining, thin diameter hyphae on the top of the cushion similar to that on the host surface, inflated granular, lightly safranin-staining hyphae in the center and dichotomously branched penetration hyphae similar in texture to those near the center of the cushion. The infection cushions adhere tightly to the host surface and appear to be cemented by a mucilagenous material (Boyle, 1921) that stains darker than surrounding material (Lumsden and Dow, 1973; Prior and Owen, 1964). This material and the dome-shape of the cushion apparently allow the cusion to exert considerable force on the cuticle to mechanically gain ingress into the host tissue by way of pore like injection pegs that form at the tips of appressoria (Abawi et al., 1975a; Boyle, 1921; Lumsden and Dow, 1973; Purdy, 1958). The mechanical entry is the probable means of entry to support by the following evidence. There is no softening, dissolution, or any modification of the cuticle prior to penetration (Boyle, 1921), infection cushions often appear to pinch the surrounding susceptible tissue into a slightly convex mound (Lumsden and Dow, 1973), the cuticle is pushed inward at points of penetration (Boyle, 1921; Lumsden and Dow, 1973) and the cuticle can remain impervious to the fungus (Lumsden and Dow, 1973) and show no alteration in staining reaction even late in pathogenesis (Boyle, 1921; Lumsden and Dow, 1973). Prior and Owen (1964) attributed the ability to penetrate lower leaves to the mechanical pressure applied by the infection cushions. However,

disintegration of the cuticle and epidermal cells which apparently result from enzyme action produced by the infection cushion takes place.

12.2 Initial Stages of Infection

After penetration of the host cuticle an inflated granular "vesicle" is formed between the cuticle and the epidermis (Boyle, 1921; Lumsden and Dow, 1973; Purdy, 1958). These vesicles give rise to "infection hyphae" that develop readily from the infection cushions and invade host tissue exclusively in an intercellular manner (Lumsden and Dow, 1973).

The penetration of host tissue by hyphae has been variously described as being intercellular and intracellular (Abawi et al., 1975a; Purdy, 1958) or indiscriminately growing in between and through cells (de Bary, 1887). In the advancing infection front, however, this is not the case. The infection process is remarkably well organized and appears to follow a sequence of events that progressively leads to total invasion and collapse of the host tissue (Lumsden and Dow, 1973). In bean tissue, infected with *S. sclerotiorum* or *S. minor* large, granular "infection" hyphae grow radically from the vesicles and develop between the cuticle and the epidermal cell layer and inter-cellularly in the cortex (Lumsden and Dow, 1973). The hyphae are quite different in size, appearance and safranin staining reaction from those seen in ordinary cultures or on the surface of the host. They resemble, in their inflated, granular appearance and light safranin staining reaction, the hyphae in the interior of infection cushions and the vesicles beneath the cuticle. The subcuticular hyphae orient parallel to one another, branch, and form an organized, fan shaped, infection front beneath the cuticle (Lumsden and Dow, 1973). The hyphae that move into the cortex develop exclusively inter-cellularly. After the initial infection period (12–24 h), the radial hyphal front's breaks up into clusters of 18–20 hyphae which become oriented parallel to the bean hypocotyl axis and develop more rapidly upward than downward or transversely. The subcuticular hyphae move more rapidly than those in the cortex and growth is more pronounced on the side of the hypocotyl on which infection occurs. Perhaps the subcuticular region offers less resistance to progress by the hyphae, thus accounting for rapid, aggressive advance up the hypocotyl, which probably is vital to successful disease development. The cortical infection hyphae complete the girdling of the hypocotyl. All hyphae to this point are large and inflated (8.5–34.0 μm, av. 19.1 μm) and penetrate tissue exclusively between cells. The infection hyphae undoubtedly are responsible for breaching the host's defenses and for initial colonization of host tissue. The infection hyphae are associated with the advancing margins of visible lesions on hosts either slightly behind the margin (Boyle, 1921; Lumsden and Dow, 1973) or slightly in advance (Abawi et al., 1975a). These hyphae probably are responsible for changes in infected host tissue. The changes include histologically detected alterations in pectic materials in cell walls two to three cells in advance of hyphae (Lumsden and Dow, 1973), death of cells in advance (Boyle, 1921; Hancock, 1972; Thatcher, 1942), copious accumulation of

fluids and water-soaking in advancing margins (Hancock, 1972; Lumsden and Dow, 1973; Newton, 1972; Thatcher, 1942), changes in permeability of cells in advance (Hancock, 1972; Newton, 1972; Thatcher, 1942) and production of enzymes and other substances responsible for pathogenicity (Dow and Lumsden, 1975; Lumsden, 1976, 1979). The primary ascospore inoculum of *S. sclerotiorum* initially infects rapeseed via petals. On young petals pathogenesis is through ascospore adhesion, germination, penetration and collapse of epidermal cells. From petals the mycelium invades leaf tissues and infection proceeds (Jamaux et al., 1995).

12.3 Advanced Stages of Infection

After colonization of host tissue by *Sclerotinia* infection hyphae, and 12–24 h after penetration, small diameter hyphal branches develop on the infection hyphae about 55 μm behind the advancing hyphal tips (Lumsden and Dow, 1973; Prior and Owen, 1964). These "ramifying hyphae" branch profusely. Extensively invade dead or dying host tissue both inter-cellularly and intra-cellularly and are capable of readily penetrating cell walls. These hyphae are considerably smaller in diameter than the infection hyphae from which they branch (Lumsden and Dow, 1973; Prior and Owen, 1964). Hyphae can be compared in size and in intensity of staining with safranin, to hyphae in culture or on the host surface (av. 8.5 μm in diameter) (Lumsden and Dow, 1973). The broad range of hyphal size from small diameter ramifying hyphae to the inflated infection hyphae can account for the extreme variability in diameter, cell length, and shape (Abawi et al., 1975a; Prior and Owen, 1964). Ramifying hyphae readily invade cells and intercellular spaces in the cortex. Hyphae also are associated with destruction of the crystalline structure of host cell walls (Calonge et al., 1969; Lumsden, 1979). Ramifying hyphae invade the vascular tissue of bean and clover (Lumsden and Dow, 1973; Prior and Owen, 1964), although with difficulty. In sunflower, *S. sclerotiorum* enters the vessels and interfascicular regions and is considered to be the cause of vascular plugging and wilting of infected plants (Pawlowski and Hawn, 1964). Crystals detected in xylem vessels may also contribute to plugging and wilting.

After extensive colonization of tissue, ramifying hyphae emerge from the host tissue, primarily through stomata or breaks in the cuticle (Lumsden and Dow, 1973). Emergence of hyphae from stomata has not been observed in potato leaves (Jones, 1976), but protruding hyphal strands are visible on the lower surfaces of bean leaves (Abawi et al., 1975a). These tufts form mycelial wefts and eventually, cottony growth on the surface of mature lesions. Sclerotial initials consisting of clumps of short, barrel shaped cells give rise to mature sclerotia in three to seven days (Abawi et al., 1975a; Lumsden and Dow, 1973). Sclerotia may form on the surface of the host in the pith, or under decaying plant parts on the soil surface. Sclerotia may not develop and mature if the food base from the infected tissue is insufficient. With the formation of sclerotia, the disease cycle is complete (Lumsden and Dow, 1973; Lumsden, 1979).

Pathogenesis is a complex, dynamic process involving the pathogen's inherent capabilities and multiple factors that govern penetration and infection of a host plant. The host plant has an array of defense mechanisms that must be breached, inactivated or annulled before disease can develop. This interaction between host and pathogen also is dependent on the surrounding environment and on time. The pathogen's battery of attacking mechanisms includes cell wall and middle lamella-degrading enzymes, toxins, enzymes to degrade host tissue and defense substances, and rapidity of infection. *S. sclerotiorum* secretes multiple pectinolytic enzymes that facilitate penetration, colonization and maceration of the plant tissues. The details of these aspects have been covered in the section of biochemistry and physiology of disease development (13, 14).

12.3.1 Sunflower

In sunflower wilt develops when mycelia from germinating sclerotia infect underground parts. Hypocotyls, especially when succulent are invaded (Hancock, 1972) but the pathogen penetrates the host mainly through the roots (Young and Morris, 1927). Huang and Hoes (1980) study of *Sclerotinia* wilt of sunflower in artificially and naturally infested soil, showed that the wilt phase results mainly from infection originating at or below the soil line. They also presented evidence to suggest that wilt phase in sunflower is caused by infection resulting from mycelial rather than carpogenic germination of sclerotia. This has been further confirmed by Huang and Dueck (1980). It was also reported that the mycelium produced, can infect root and hypocotyls of sunflower seedlings without the addition of nutrients or wounding of the host tissues. This is contradictory to the earlier reports on beans that an exogenous source of energy is required for successful infection by mycelium from *S. sclerotiorum* (Abawi and Grogan, 1975). It has been observed that the penetration site on the tap root is within the zone of lateral roots (Huang and Hoes, 1980). Ultimately, it reaches the tap root and continues to grow downward and upward, disintegrating the parenchymatous and cortical tissues. The rot spreads simultaneously to other roots of the same plant, either by contact or presumably via points of root attachment along the tap root and the lower hypocotyls. As the infection progresses, the pathogen also reaches the pith of the tap root and expands further upward and into the pith of the stem. The plants at the site of infection develop wilt first and become the primary infection locus from which the fungus spreads by root contact from plant to plant in a sequential order (Huang and Hoes, 1980).

Under conditions of cool temperatures and high humidity sclerotia may germinate indirectly by producing apothecia with subsequent release of ascospores which then cause sunflower stalk or head rot (Hoes and Huang, 1976; Huang and Dueck, 1980). Localized symptoms on old plants confirm that ascospores are the source of infection in the field under Indian and Iran conditions (Kolte, 1985a; Ale-Agha, 1974). Development of flower infection independent of infection resulting in wilt has also been reported from Australia (Krexner, 1969). The ascospores can infect

both healthy and wounded tissues (Antokolskaya, 1927). In both phases, the wound may not be essential for infection (Antokolskaya, 1927; Huang and Dueck, 1980), but it is reported that the wound caused by mechanical agencies or wounds caused when roots emerge from the pericycle rupturing the cortex favour the development of infection. Birds appear to carry mycelium from diseased heads or from infested soil to wounds which they scratch in the healthy seeds (Young and Morris, 1927). In most of the sunflower production areas, both phases of infection occur, but generally the wilt phase is more prevalent than the head rot phase. Cuk (1978) observed that in sunflower leaf infections usually occur in a region close to the petiole and that the ninth and tenth pair of leaves are most frequently infected, usually at a time between the appearance of the bud and the flowering stages of plant growth. According to Sedun and Brown (1987) ascospores of *S. sclerotiorum* infect fully expanded sunflower leaves in the absence of added nutrients, wounds or senescent tissue. The infection is confined to a specific region around the junction of the leaf blade and the petiole and is associated with sites of sucrose secretion by the host. In these areas ascospores germinate and form extensive colonies on the leaf surface. Simple appressoria are visible at 24 h and complex appressoria at 48 h after inoculation. In sunflower, pathogenesis proceeds in essentially the same way, whether the inoculum is hyphae from ascospores or hyphae from sclerotia that germinate directly. The progress of invading hyphae is facilitated by extra cellular pectolytic enzymes that digest middle lamella of sunflower cells containing pectic substances. High polygalacturonase activity and considerable losses of pectic acid are reported to be associated in sunflower infected by *S. sclerotiorum* (Hancock, 1966). The pH of the infected tissue decreases from 6.2 to 4.5, which is favourable to polygalacturonase activity. Activity of pectin methylesterase is increased, but neither pectin transeliminase nor polygalacturonase transeliminase is detected in significant amount (Hancock, 1966). Similarly, xylanase and arbanase systems (hemicellulases) are associated with hypocotyl infection (Hancock, 1967). Pawlowski and Hawn (1964) reported that sunflower wilt due to *S. sclerotiorum* is not due to production of transportable toxin but due to mechanical plugging of vessels. However, the work of Huang and Dorrell (1978) shows that wilting can be induced by sterile culture filtrate without direct involvement of the pathogen. According to them, the toxic metabolite plays an important role in the development of disease symptoms, as culture filtrate of the fungus has been found to be toxic to sunflower seedlings causing wilt symptoms similar to those developed on naturally infected plants. The toxic substance appears to be oxalic acid. Sunflower leaves from wilted plants have been reported to show ten times more oxalic acid than leaves of healthy plants (Noyes and Hancock, 1981). Oxalic acid has been shown to move systemically in the plant and accumulate to critical levels and this elicits the wilt syndrome (Noyes and Hancock, 1981). Cells of sunflower plants, just above the lesion induced by the fungus, show less leakage of electrolytes and less permeability to water and urea compared with corresponding cells in healthy plants (Hancock, 1972). Infected plants contain more soluble sugar than the healthy plant (Polyakov, 1973). After inoculating with ascospores of *S. sclerotiorum*

in tolerant variety HA 302, there is cell collapse, changes in cell wall composition and an increase in phenolic compounds in the tissues of corolla and style which prevents the pathogen from advancing. This response is weaker in susceptible variety HA 891 (Rodriguez et al., 2004).

12.3.2 Rapeseed-Mustard

In oilseed rape crops infection by *Sclerotinia* is usually via ascospore bearing petals. At petal fall ascospore infected petals stick to leaves initiating infections that can develop into stem rot lesions (Mc Cartney et al., 2001a, b). Ascospores discharged from the apothecia at the base of the plants in soil constitute an important primary source of infection. Mycelium in soil or mycelium arising from the sclerotia is less important initial source of infection because of the low competitive saprophytic ability of the fungus (Newton and Sequeira, 1972a). The ascospores can germinate in the presence of a thin film of water, in less than 24h at 5–30°C, with an optimum range being 5–10°C. On germinating, the ascospores give rise to infection hyphae and initial penetration of the host tissue takes place directly by mechanical pressure through the cuticle or the infection hypha may penetrate already wounded or injured tissue also. After entrance of the fungus into the host, the mycelium ramifies intercellularly or intracellularly causing colonization of tissue primarily because of enzymatic dissolution of the cell wall in advance and cells die some distance ahead of the invading hyphae. Pectolytic enzymes are responsible for tissue maceration indirectly damaging the cell membrane, which results in subsequent death of cells (Morrall et al., 1972). Rai and Dhawan (1976a) and Dhawan and Srivastava (1987) reported production of polymethyl galacturonase transeliminase (PGTE) and cellulase (CX) enzymes by *S. sclerotiorum* infecting rapeseed-mustard plants. According to them, virulence of different isolates appears to be associated with the activity of PMG and CX enzymes. The role of protease activity in infection of plants of *B. juncea* is also demonstrated (Dhawan, 1980). It appears that invasion of tissues of *B. juncea* is also related to the infection process, mediated by production of a toxin which is identified as oxalic acid (Rai and Dhawan, 1976a), which is formed in infected *B. juncea* plants. The toxic substance is thermostable, translocatable and treatment of the host plant with culture filtrate results in infection.

Pathogenesis of *S. sclerotiorum* is complex and not well understood (Dickman and Mitra, 1992). The fungus produces a wide array of degradative, lytic enzymes (e.g., endo and exopectinases, cellulases, hemicellulases, proteases) which are believed to facilitate colonization and host cell wall degradation (Marciano et al., 1983; Riou et al., 1991). Infection of canola pollen by *S. sclerotiorum* occurs by direct hyphal penetration of cell walls without the formation of appressoria or infection cushions. Hyphal penetration is more commonly observed through the germinative pores than other parts of pollen walls. Plasmolysis and disintegration of the pollen cytoplasm occurs as a result of hyphal ramification within the infected pollen grains (Huang et al., 1998).

12.3.3 Carrot

Infection of carrot by *S. sclerotiorum* occurs more readily when mycelial inoculum is placed near foliage which is in contact with the soil than when mycelial inoculum is placed near the roots. Infection of the roots occurs only after the crown and foliage become infected. Direct root infection by mycelium arising from sclerotia in the soil is therefore unlikely. Mycelial inoculum placed on the soil near the crown readily causes disease symptoms in greenhouse grown carrots but leaf wetness is essential for 11 days for foliar applied ascospores to induce disease on the foliage and in stored roots. Field grown plants that receive mycelial inoculum develop disease symptoms while plants treated with ascospores do not (Finlayson et al., 1989).

12.3.4 Alfalfa

Examination of ascospore-pollen mixtures incubated at room temp. (20–22°C) for five days reveals that numerous pollen grains are infected by *S. sclerotiorum* by direct hyphal penetration through the equatorial germinative pores or through the exine and intine layers of the pollen wall without the formation of infection cushions or appressoria. After penetration, hyphae ramify within the pollen grains, causing plasmolysis of the cytoplasmic membrane and eventual disintegration of the pollen cytoplasm. It is suggested that Lucerne pollen may play a role in the epidemiology of blossom blight in Lucerne (Huang et al., 1997b).

12.4 Genes Associated with Fungal Pathogenesis

To identify genes involved in fungal development and pathogenesis, Li-Ru Gang et al. (2004a, b) generated 2,232 expressed sequence tags (ESTs) from two cDNA libraries constructed using either mycelia grown in pectin medium or tissues from infected *Brassica napus* stems. A total of 774 individual fungal genes are identified of which 39 are representing only among the infected plant EST collection. Annotation of 534 unigenes is possible following the categories applied to *Saccharomyces cerevisiae* and the Universal Gene Ontology scheme. cDNAs are identified that encodes potential pathogenicity factors including four endopolygalacturonases, two exopolygalacturonases and several metabolite transporters. The potential role of these genes, as well as those encoding signal transduction factors, in the infection process is very vital.

Five major and several minor PG isoenzymes have been identified in a *Sclerotinia sclerotiorum* isolate from *Brassica napus* by iso-electric focusing and pectin gel overlays. Using a combination of degenerate PCR and expressed sequence tags (ESTs) four endo-polygalacturonase (PG) genes, designated as

sspg1d, *sspg3*, *sspg5*, and *sspg6* and two exo-PG genes, *ssxpg1* and *ssxpg2* are identified. *SSPG1d* is a member of the PG gene family previously described by Fraissinet-Tachet et al. (1995). The mature *SSPG1d* is a neutral PG, whereas fully processed *SSPG3*, *SSPG5*, and *SSPG6* are acidic enzymes. Under saprophytic growth conditions, *sspg1d*, *sspg3*, *sspg5* and *ssxpg1* expression is induced by pectin and galacturonic acid and subject to catabolite repression by glucose. Conditions could not be identified under which *sspg6* or *ssxpg2* are expressed well. Transfer of mycelia from liquid media to solid substrates induces expression of sspg1d suggesting that it may also be regulated by thigmotrophic interactions. Under pathogenic conditions, *sspg1d* is highly expressed during infection. *sspg3* is also expressed during infection, albeit at lower levels than sspg1d, whereas *sspg5*, *sspg6* and *ssxpg1* are expressed only weakly (Li-Ru Gang et al., 2004a, b).

12.5 Pathogenic and Saprophytic Phases of *Sclerotinia*

Sclerotinia sclerotiorum is unusual among necrotrophic pathogens in its requirement for senescent tissues to establish an infection and to complete the life cycle. A model for the infection process has emerged whereby the pathogenic phase is bounded by saprophytic phases; the distinction being that the dead tissues in the latter are generated by the actions of the pathogen. Initial colonization of dead tissue provides nutrients for pathogen establishment and resources to infect healthy plant tissue. The early pathogenicity stage involves production of oxalic acid and the expression of cell wall degrading enzymes, such as specific iso-forms of polygalacturonase (*SSPG1*) and protease (ASPS), at the expanding edge of the lesion. Such activities release small molecules (oligogalacturonides and peptides) that serve to induce the expression of a second wave of degradative enzymes that collectively bring about the total dissolution of the plant tissue. Oxalic acid and other metabolites and enzymes suppress host defenses during the pathogenic phase, while other components initiate host cell death responses leading to the formation of necrotic tissue. The pathogenic phase is followed by a second saprophytic phase, the transition to which is effected by declining cAMP levels as glucose becomes available and further hydrolytic enzyme synthesis is repressed. Low cAMP levels and an acidic environment generated by the secretion of oxalic acid promote sclerotial development and completion of the life cycle (Hegedus and Rimmer, 2005).

12.6 Seed Infection

Sclerotinia infected or infested seeds have been reported in several crops including sunflower (Young and Morris, 1927), safflower (Chaudhary and Putoo, 1991), cabbage, cauliflower, kale (Neergaard, 1958), clover (Scott, 1981b; Scott and Evans, 1984), beans (Starr et al. 1953; Akai, 1981), lupine (Chamberlain, 1932), peanut

(Porter and Beute, 1974; Akem and Melouk, 1990), rape, barley (Adams and Ayers, 1979), wheat, sorghum, flax (Miclaus et al., 1988) and soybean (Anderson, 1985), the role of these seeds in the dissemination of *Sclerotinia sclerotiorum* and in the epidemiology of the disease has not been carefully investigated. Whether *S. sclerotiorum* is transmitted by seed is an important question, especially in seed production areas free of the disease. Nobel and Richardson (1968) listed white mould of bean as a seed-borne disease and transmission of the fungus by or with bean and other types of seed has been reported (Baker and Davis, 1951; Blodgett, 1946; Hungerford and Pitts, 1953; Nicholson et al., 1972). However, according to three years observation of Steadman (1975) seed transmission of *Sclerotinia* in Nebraska bean fields could not be established. *S. sclerotiorum* has been isolated from 48 per cent of seed lots of bean cv. Great Northern and Pinto harvested from white mould infected plants, but from only 6 per cent of seed lots harvested from healthy appearing plants in Western Nebraska. The fungus has been recovered from less than 0.5 per cent of normal seed, but from nearly 12 per cent of chalky, discoloured and shrivilled seed. Infected lots of seed planted in sterilized soil in a greenhouse humidity chamber did not produce white-mould infected bean plants. Seeds infected with *S. sclerotiorum* did not germinate *in-vitro. S. sclerotiorum* can be disseminated with seed, but this is unlikely to be of epidemiological significance. Hungerford and Pitts (1953) reported that a small number of plants are infected with white mould when seed from *S. sclerotiorum* infected Pinto bean fields in Idaho is planted in the greenhouse. According to Tu (1988), *S. sclerotiorum* survives in infected seeds of white bean as dormant mycelium in testa and cotyledons. The rate of survival is 85–89 per cent and does not change over a three year period. When the infected bean seeds are sown in soil or sand, 88–100 per cent fail to germinate and rot. In place of each seed, three to six sclerotia are formed. A low percentage of these sclerotia germinate carpogenically with or without preconditioning (2.5 and 11.5 per cent respectively). In soybean, incidence of seed borne *S. sclerotiorum* infection from discoloured shriveled seeds ranges from 0 to 70 per cent (Hartman et al., 1998).

Myceliogenic germination of sclerotia with and without preconditioning is 35.5 and 70.5 per cent on water agar and 81–93 per cent on glucose agar, respectively. Both preconditioning and non preconditioning sclerotia which are scattered on soil surface can germinate myceliogenically and infect bean leaves by contact. It is therefore, concluded that dormant mycelia in the infected seeds play an important role not only in dissemination of the fungus but also in epidemiology of the disease. These results contradict previous reports that sclerotia placed in direct contact with bean tissues failed to infect even after prolonged incubation under optimum conditions (Abawi and Grogan, 1975, 1979). Infected seed may increase the inoculum potential of the pathogen in soil. Under favourable conditions sclerotia are formed on infected seeds and may be capable of producing apothecia later in the same season (Hungerford and Pitts, 1953).

Chapter 13
Biochemistry of Host-Pathogen Interaction

The cell wall degrading enzymes capable of destroying cellular components and production of oxalic acid are associated with disease development. de Bary (1887) stated "The power of infecting is shown by the power of penetrating the membranes (cuticle) which are evidently dissolved at the point of penetration. Hence, it is very probable that this power depends on the presence of a substance which can dissolve a membrane, ferment (enzyme) in fact." In addition, Prior and Owens (1964) mentioned possible enzymatic action on the cuticle in association with infection cushions on clover leaves. However, Purdy (1958) and Boyle (1921) were unable to discern any alteration of host tissue due to the diffusion of substances before penetration. Until the possibility of enzymatic action on host tissue prior to penetration is demonstrated experimentally, ingress into the host must be considered a mechanical process as based on histological evidence.

Pectolytic enzymes are always associated with diseases caused by *Sclerotinia* (Barkai- Golan, 1974; Calonge et al., 1969; Echandi and Walker, 1957; Hancock, 1966; Held, 1955; Lumsden, 1976, 1979; Maxwell and Lumsden, 1970; Morrall et al., 1972; Newton, 1972; Riou et al., 1991; Sharma and Sharma, 1984a; Van den Berg and Yang, 1969). Qualitative and quantitative information is rather limited, but these enzymes have been associated with quantitative decreases in the pectic substance content of diseased tissue (Hancock, 1966) and with histochemical and structural changes in host cell middle lamellae (Calonge et al., 1969; Lumsden, 1976). In addition, pectolytic enzyme activity has been localized in infected tissue. Several pectolytic enzymes are produced in diseased bean tissue and similar ones appear to be produced in cultures of *S. sclerotiorum* (Lumsden, 1976). Lumsden (1976) detected a viscosity-reducing polygalacturonase (PG) as early as 12 h after inoculation of bean plants. The activity reaches a peak 24 h after inoculation at about the time of irreversible establishment of the infection. Thereafter, the activity decreases (up to 48 h), until in maturing lesions, another peak of viscosity reducing activity occurs. The first peak of enzyme activity has been identified as an endopolygalacturonase (endo-PG), based on random hydrolysis of the pectin polymer substrate (Hancock, 1966; Lumsden, 1976). The PG is adaptive, that is glucose suppresses its formation when added to growth media (Lumsden, 1976; Lumsden and Dow, 1973). Apparently, hydrolysis products in diseased tissue are suppressive,

G.S. Saharan, Naresh Mehta, *Sclerotinia Diseases of Crop Plants: Biology, Ecology and Disease Management*,

since the enzyme activity declines after the initial 24 h period of disease incubation (Lumsden, 1976). The optimum pH for activity of the enzyme is between pH 4.5 and 5.5. In addition, the enzyme appears to be more active on pectate than on pectin. The endo-PG is localized in advancing margins of infected bean hypocotyls during the early (up to 48 h) stages of infection but not during the later stages and is not associated with mycelium in culture (Lumsden, 1976). The location of activity has been determined in thin sections of diseased tissue by plating them on a pectate medium in which opaque halos surround those tissue sections with intense enzyme activity. In contrast to the intense reaction of the endo-PG, pectinase activity in older lesions produces a hazy reaction when tissue slices are placed on the pectate medium. This enzyme catalyzes the rapid release of reducing endogroups and completely hydrolyzes pectin substrate to galacturonic acid (Lumsden, 1976). Although possibly a mixture of exo-PG and endo-PG, the predominant activity is characteristic of exo-PG. The activity is associated with advancing margins of maturing lesions with older portions of lesions and with mycelium in older cultures. The enzyme activity is comparable to that described by Hancock (1966) in two to four days-old infected sunflower and in other host tissue assayed for enzyme activity after several days of incubation (Barkai-Golan, 1974; Morrall et al., 1972; Newton, 1972). Production of this enzyme is not suppressed by glucose (Lumsden, 1976). The optimum pH for activity is in the range of pH 4.5 to 5.5 (Echandi and Walker, 1957; Hancock, 1966; Lumsden, 1976). The production of polygalacturonases and pectinases from *S. sclerotiorum* is induced by galacturonic acid from plant cell walls (Fraissinet-Tachet and Fevre, 1996a).

Pectin methyl esterase (PME) also has been detected early in pathogenesis in diseased bean tissue and is associated with the advancing margins of lesions throughout disease development (Lumsden, 1976). The fungal PME is clearly distinguishable from host PME on the basis of its lack of dependence on salt for activation and its much lower optimum pH (pH 5.0) for activity than the host PME (pH 8.0). The PME has been demonstrated in diseased tissue by others (Barkai-Golan, 1974; Hancock, 1966; Morrall et al., 1972) and its action in the demethylation of host plant pectin has been clearly demonstrated (Hancock, 1966; Lumsden, 1976). Pectin transeliminase is not produced by *Sclerotinia* spp. (Barkai-Golan, 1974; Hancock, 1966; Lumsden, 1976; Morrall et al., 1972). Purification and characterization of extracellular pectinolytic enzymes has been done (Riou et al., 1992).

Cellulase and hemicellulase enzymes often have been associated with *Sclerotinia* spp. and pathogenesis (Barkai-Golan, 1974; Bauer et al., 1977; Calonge et al., 1969; Hancock, 1967; Lumsden, 1969; Newton, 1972; Riou et al., 1991; Sharma et al., 1983). Their role in pathogenesis, however, has not been elucidated. Sequential degradation by *S. sclerotiorum* of native, insoluble cellulose is attributed to C1 enzyme from *S. sclerotiorum*, soluble cellulose by Cx enzyme, and hydrolysis of cellobiose to glucose by β-1-3-glucosidase (Lumsden, 1969). The optimum pH for Cx enzyme activity is pH 3.0. If this series of enzymes is operational in the degradation of native cellulose, *S. sclerotiorum* appears to have the complete system and can utilize native cellulose as an energy source. Abundant cellulase is produced adaptively in diseased tissue (Barkai-Golan, 1974; Lumsden, 1969). Moreover,

the content of a cellulase in diseased tissue declines substantially with the age of an infection (Lumsden, 1969) and alteration of cellulose structure in infected tissue has been observed (Boyle, 1921; Calonge et al., 1969). Similarly, the araban and galactan fractions of infected sunflower tissues are degraded extensively (Hancock, 1967). Arabanase is associated with infected tissue, but galactanase activities are not measured. In contrast, xylanase is detected in infected tissues at concentrations capable of extensively degrading native xylan, however, xylan breakdown appears to be restricted. This suggests that arabans and galactans are more accessible to enzymatic breakdown than xylans. Partially purified galactanase (Bauer et al., 1977) readily solubilizes carbohydrates, including the galactan component of sycamore and potato cell walls. It does not macerate potato tuber tissue, although galactose is released. Besides cell wall-degrading enzymes, a few other enzymes have been studied in relation to pathogenesis. Phosphatidase β, which is capable of hydrolyzing phosphatide components of cell membranes is produced abundantly in culture and is detectable early in disease development in bean (Lumsden, 1970; Newton, 1972). The enzyme is inductive, extracellular, activated by calcium, and has an activity optimum at pH 4.0.

Proteolytic enzyme activity, potentially responsible for degradation of host protoplasm and possibly cell wall constituents is detected in *S. sclerotiorum* and *S. minor* cultures (Khare and Bompeix, 1976) and in infected tissue (Khare and Bompeix, 1976; Newton, 1972). In diseased celery, cucumber and carrot extracts, notable protease activity is detected at two days after inoculation and increases to a maximum at ten days (Khare and Bompeix, 1976). The optimum pH for protease activity is 3.0.

13.1 Molecular Aspects of Host-Pathogen Interaction

Research on molecular aspects of biotrophic pathogenicity often concentrates on the fine-tuned interaction between a pathogen and its, sometimes even single host (Basse and Steinberg, 2004; Thomma et al., 2005). By contrast for many necrotrophic pathogens that often have a broad host range, research on the molecular aspects of pathogenicity is mainly concentrated on the contribution of hydrolic enzyme activity and production of certain metabolites that can act as toxins (Kars and van Kan, 2004; Thomma, 2003; Toth et al., 2003). This has been the major focus for *S. sclerotiorum* as well with emphasis on the role of cell wall degrading enzymes (CWDEs) and oxalic acid.

13.2 Cell-Wall Degrading Enzymes

Plant pathogenic fungi can facilitate colonization of their hosts by the production of a wide array of CWDEs, including pectinases, β-1, 3 glucanases, glycosidases, cellulases, xylanases and cutinases (Annis and Goodwin, 1997). Many of these CWDEs

can occur in multiple isozymes that differ in isoelectric point, molecular weight, processing or transcriptional regulation (Keon et al., 1987), giving great flexibility to the pathogen for penetration and colonization of the host and facilitating the creation of a pool of assimilable nutrients. In culture, expression of most CWDEs is tightly regulated at the transcription level by the availability of carbon and/or nitrogen sources (Alighisi and Favaron, 1995). Such a carbon catabolite repression mechanism permits the fungus to adopt its metabolism to the availability of glucose or other carbon sources it can metabolize. In addition, ambient pH can also regulate enzyme levels at the transcription level (Cotton et al., 2003; Rollins and Dickman, 2001).

During the interaction with its host, *S. sclerotiorum* secretes a full complement of CWDEs (Table 13.2.1) that can facilitate penetration, macerate tissues and degrade plant cell wall components (Hancock, 1966; Lumsden, 1969; Riou et al., 1991). Pectin is a major constituent of the plant cell wall and pectinases produced by *S. sclerotiorum* play a role in pectin degradation. Pectin hydrolysis weakens the cell wall to facilitate penetration and colonization of the host while also providing the fungus carbon sources for growth. *S. sclerotiorum* produces several forms of pectinolytic enzymes that are capable of killing plant cells and macerating plant tissues on their own (Alighisi and Favaron, 1995), suggesting a role in pathogenicity.

Table 13.2.1 Genes encoding cell wall degrading enzymes (CWDEs) in *Sclerotinia sclerotiorum* (Adapted from the publication of Bolton et al., 2006. With permission)

Gene/protein designation	Accession no.	Reference
Acidic endoPGs		
PG2	S13661	Waksman et al. (1991)
PG3	B60155	Waksman et al. (1991)
sspg3	AY312510	Li et al. (2004a)
pg5	Y13669	Kasza et al. (2004)
sspg5	AY496277	Li et al. (2004a)
pg6	AJ539086	Kasza et al. (2004)
pg7	AJ539087	Cotton et al. (2003)
PGa	CAF05669	Favaron et al. (2004)
Neutral or basic endo PGs		
pg1	L12023	Reymond et al. (1994)
pg2	L29040	Fraissinet-Tachet and Fevre (1996)
pg3	L29041	Fraissinet-Tachet et al., (1996)
sspg1d	AF501307	Li et al. (2004a)
sspg6	AF501307	Li et al. (2004a)
PGb	CAF05670	Favaron et al. (2004)
Exo PGs		
ssxpg1(partial cDNA)	AY312511	Li et al. (2004a)
ssxpg2 (partial cDNA)	AY312512	Li et al. (2004a)
Non-aspartyl acid protease		
acp1	AF221843	Poussereau et al. (2001a)
Aspartyl protease		
acpS	AF271387	Poussereau et al. (2001b)

The polygalacturonases (PGs) are important pectinases that can degrade unesterified pectate polymers, the structural polysaccharides found in the middle lamella and the primary cell wall of higher plants. PG activity in *S. sclerotiorum* is induced by pectin or pectin monomers, such as galacturonic acid, but is repressed by the presence of simple sugars (Fraissinet-Tachet and Fevre, 1996a; Riou et al., 1992), PGs have been implicated as virulence factors through targeted gene disruption in a few pathosystem (Garcia-Maceira et al., 2001; ten Have et al., 1998; Kars et al., 2005; Shieh et al., 1997; Wagner et al., 2000) but conclusive evidence for a universal role in virulence is lacking (Gao et al., 1996; Scott-Craig et al., 1990).

Endo PGs are endo-acting enzymes that catalyse the hydrolysis of homogalacturonan while exoPGs cleave monomeric or dimeric glycosyl groups from the pectic cell wall polysaccharides, resulting in substrate fragmentation and the release of potential nutrients (Kars and van Kan, 2004). A number of both endo- and exo PGs have been cloned and characterized in *S. sclerotiorum* (Table 13.2.1).

Molecular genetics have shown that fungi can produce multiple forms (isozymes) of endo PGs through post translational (glycosylation) or post secretional (proteolysis) modifications, or alternatively are derived from a multigene family (Bussink et al., 1992; Caprari et al., 1993; Fraissinet-Tachet et al., 1995). A recent study on five endo PGs from the closely related necrotroph *Botrytis cinerea* showed that these enzymes differ in biochemical properties and necrotizing activity on different host plants (Kars et al., 2005). *S. sclerotiorum* also secretes a number of molecular forms of pectinases that exhibit the same enzyme activity (Riou et al., 1991). Several *S. sclerotiorum* purified acidic endoPGs display differences in isoelectric point but have a similar molecular weight, suggesting that differential glycosylation (which will affect the molecular mass without modifying the net charge of proteins) may have little influence on PG multiplicity in *S. sclerotiorum* (Waksman et al., 1991). Fraissinet-Tachnet et al. (1995) demonstrated that the multiplicity of pectinolytic enzymes and polygalacturonase isozymes of *S. sclerotiorum* are encoded by a multigene family comprising seven members and constituting two subfamilies. Although the advantages of multiple copies of pectinase genes have yet to be demonstrated experimentally for this fungus, one can speculate that multiple copies of functionally related genes confer flexibility and adaptability to pathogen with such a wide host range. Further more analyses of *S. sclerotiorum* endo PGs have shown differential expression during pathogenesis (Cotton et al., 2002; Kasza et al., 2004; Li et al., 2004b). In a recent study by Kasza et al. (2004), Northern blot analysis revealed endoPGs *pg1*, *pg2* and *pg3* which are expressed maximally during the phase of colonization of healthy plant tissues at 36 h post inoculation (hpi), but are not detectable at 96 hpi. RT-PCR show *pg6* and *pg7* which are detected from 24 hpi until the end of the time course experiment at 96 hpi, but *pg5* transcripts are only detected between 48 and 72 hpi which correlates with the final phase of maceration. The depletion of inducers such as polygalacturonic acid during the course of infection, cabolite repression by accumulation of end products or an acidic ambient pH may create a sequence of environmental conditions that act to activate or repress transcription of individual endoPG gene (Kasza et al., 2004).

Plants are known to produce cell wall associated glycoproteins that are capable of inhibiting fungal endoPGs called (PGIPs) polygalacturonase–inhibiting proteins (De Lorenzo et al., 2001). PGIP isolated from soybean can display differential and inhibitory activity towards *S. sclerotiorum* endoPGs (Favaron et al., 1994, 2004). Oligogalacturonides released from the plant cell wall by the enzymatic activity of endoPGs have been shown to acts as endogenous elicitors of the hypersensitive response (HR) (Davis et al., 1986). *Phaseolus vulgaris* PGIP was shown to prevent the HR induced by *S. sclerotiorum* endoPGs (Zuppini et al., 2005).

The oxidative burst is an early plant defense reaction that results in the formation of reactive oxygen species such as H_2O_2 and O_2 which coincide with the HR to sequester pathogen attack (Wojtaszek, 1997). Although the HR is generally thought to prevent colonization of biotrophic pathogens, the virulence of necrotrophic fungi like *S. sclerotiorum* and *Botrytis* species that obtain nutrients from necrotic host tissues might actually be strengthened by the HR (Govrin and Levine, 2000; Thomma et al., 2001). Plants unable to incite HR shows increased resistance to *S. sclerotiorum* in tobacco as well as *Arabidopsis thaliana* (Dickman et al., 2001; Govrin and Levin, 2000).

Non-pectinolytic CWDEs have received less attention in *S. sclerotiorum* (Table 13.2.1) However, enzymes such as proteases, cellulases and glucoamylases have been characterized for this fungus (Lumsden, 1969; Martel et al., 2002; Poussereau et al., 2001a, b; Riou et al., 1991). As up to 10 per cent of the plant cell wall consists of proteins (Carpita and Gilbeaut, 1993), proteases may be an important aspect of *S. sclerotiorum* pathogenesis.

13.3 Cloning and Sequence Analysis of A Polygalacturonase-Encoding Gene from *Sclerotinia*

Sclerotinia sclerotiorum produces a number of extracellular pectin-degrading enzymes. The complete sequence of a gene (*pg1*) encoding an endopolygalacturonase (*PG1*) has been cloned and determined. The coding region consisted of a non-interrupted 1,143-bp open reading frame. *S. sclerotiorum pg1* was compared with other fungal PG-encoding genes. Basic transcription control sequences were identified in the five non coding regions. The deduced amino acid (aa) sequence (380 aa) of the enzyme was compared with seven fungal PG sequences and showed a high level of identity (41.5 to 59.8 per cent). Predicted secondary structures were compared, revealing a similar protein organization most probably in antiparallel beta sheets. Hybridization analysis using a *PG1* 0.65-kb *BamHI* fragment as a probe allowed the identification of seven different recombinant phages from a genomic library. Analysis of the hybridizing restriction fragments suggested that PG-encoding genes are organized as a family (Reymond et al., 1994).

Chapter 14
Physiology of Host-Pathogen Interaction

The knowledge gained through histopathology, ultrastructural changes and host-pathogen interaction gives indirect insight into the physiological processes that occur during pathogenesis. The actual study of the physiological processes provides direct evidence necessary to establish the mechanisms of pathogenesis.

14.1 Colonization of Tissue

Exclusive intercellular penetration of infection hyphae through tissue (Lumsden and Dow, 1973) is enhanced by enzymes capable of degrading the middle lamella of host cells. The three pectolytic enzymes produced by *Sclerotinia* spp. serves the pathogen in this capacity. The endo-PG (Lumsden, 1976) undoubtedly is essential for successful advance of the pathogen during the very early stages of pathogenesis. After demethylation of pectin by PME, endo-PG probably is responsible for hydrolysis of the middle lamella of cells, thus enabling the fungus to move rapidly through tissues in an intercellular manner. Indirect evidence also suggests an important role for endo-PG. The ability to produce large quantities of the enzyme *in vitro* is associated with isolates of *S. sclerotiorum* that are most virulent on bean (Lumsden, 1976). In addition, the endo-PG readily macerates susceptible bean and cucumber tissue (Lumsden, 1976) but not resistant (Echandi and Walker, 1957; Held, 1955) potato tuber tissue (Lumsden, 1976). The early appearance and subsequent inactivation of this enzyme in diseased tissue may account for the lack of correlation of PG with virulence (Held, 1955; Morrall et al., 1972; Newton, 1972). The PME probably is essential for rapid action by endo-PG. These enzymes work together to degrade highly methylated pectin. The PME demethylates pectin in the middle lamella, forming pectate, which is the preferred substrate for *Sclerotinia* exo and endo-PG (Hancock, 1966; Lumsden, 1976). Correlation of PME with virulence of *Sclerotinia* isolates has not been possible, however (Lumsden, 1976; Morrall et al., 1972), PME also is active during the later stages of pathogenesis at which time the exo-PG is most active. Exo-PG hydrolyzes pectate more readily than pectin and, therefore exo-PG and PME also work in concert to degrade middle lamellar pectin. The production of exo-PG is correlated with growth of

G.S. Saharan, Naresh Mehta, *Sclerotinia Diseases of Crop Plants: Biology, Ecology and Disease Management*,

Sclerotinia (Lumsden, 1976) and also may play a role in the nutrition and development of the pathogen in invaded tissue.

14.2 Nutrition During Pathogenesis

The nutrition of *Sclerotinia* spp. during all stages of disease development is probably the most important factor in determining success or failure in the establishment of disease in the host. Even before infection, the availability of a food base is usually a prerequisite for successful infection (Abawi et al., 1975b; de Bary, 1887; Lumsden and Dow, 1973; Purdy, 1958). During infection, the fungus organizes into specialized infection hyphae, which must require a considerable amount of energy and in turn, an abundant, readily available source of nutrients. The nutrition provided by the food base may determine whether or not disease occurs on a potential host. Infection hyphae of *S. sclerotiorum* can be induced in culture when the fungus is grown on cellophane placed on an appropriate agar medium (Lumsden, 1975). Production of the inflated, parallel infection hyphae depends on the nutrient status of the medium and physical contact with the surface of the cellophane film. Cellophane covered bean stem extract medium induces a greater amount of parallel hyphal arrangement and more inflated hyphae than cellophane covered cornmeal agar. In general, media made from host plant tissues induce a greater amount of infection hyphae formation than nonhost media (Lumsden, 1979). Cellulase, hemicellulase, exo PG, phosphatidase, proteolytic enzymes and other enzymes may play a nutritional role in pathogenesis. The action of these enzymes on cell walls and cell contents can provide an abundant carbon and nitrogen supply essential for the intensive metabolic activity of *Sclerotinia* spp. as the infection hyphae move rapidly through host tissue (Lumsden, 1979). Cell-wall degrading enzymes (Bauer et al., 1977; Hancock, 1967; Lumsden, 1969, 1976) possibly produced by the ramifying hyphae of *Sclerotinia* spp. (Lumsden and Dow, 1973) can be responsible for extensive degradation of cell walls and thus make abundant carbohydrates available. The ramifying hyphae which branch from infection hyphae well behind the hyphal tips, clearly are capable of intracellular colonization of host cells, thus cellulolytic enzymes capable of degrading cell walls must be produced to allow penetration by hyphae. Further evidence suggests a secondary or nutritional role of cell wall-degrading enzymes in pathogenesis. The decrease in a cellulose content of infected tissue is slight two days after inoculation when disease is clearly established, but becomes extensive later in pathogenesis (Lumsden, 1969). Hemicellulose degradation also is extensive late in pathogenesis (Hancock, 1967). In addition examination of infected tissue reveals no alteration in the birefringence of infected host tissue early in pathogenesis or at the margin of lesions, but destructin of birefringence later in disease development. This suggests alteration of the cystalline structure of cell walls in tissues after colonization by *Sclerotinia* infection hyphae (Lumsden, 1979). The total soluble protein content of the resistant cvs is higher than less resistant cvs (Kamara et al., 1991).

Nutritional sources of nitrogen required for growth and extracellular enzyme production can be supplied by the action of phosphatidase (Lumsden, 1970; Newton, 1972) and proteases (Khare and Bompeix, 1976). A specific nutritional role for these enzymes cannot, however, is assigned until further work establishes such a role. The contents of invaded cells also supply nitrogen. *S. sclerotiorum* appears to utilize organic or ammonical forms of nitrogen more rapidly than nitrate (Held, 1955). Hydrolyzed plant material in the killed portions of invaded tissue is probably the primary source of nutrients. Thatcher (1942), however, suggested that changes in cell permeability in advance of invading hyphae may satisfy food requirements during the initial period of infection before hydrolysis of cell wall material and death of protoplasts.

14.3 Permeability Changes and Water Relationships

Increased permeability of infected host cells has been assumed since the classical work of Thatcher (1942). Fourfold increases in permeability of infected tissue are detected in detached celery petioles. In addition, the permeability changes are noted “inches away” from any sign of necrosis. These changes in permeability are considered responsible for the water-soaking symptoms of infection and death of cells. Hancock (1972) gives another interpretation of permeability changes in *Sclerotinia*-infected tissue. The study reveals that permeability as indicated by influx and efflux of water and urea and electrolyte leakage is less in sunflower hypocotyl sections from above lesions caused by *S. sclerotiorum* than comparable sections from healthy plants. Decreased permeability of host cells above lesions is thought to be associated with changes in non-lipid components of the plasmalemma. In addition to these findings, Newton (1972) was unable to establish a cause-effect relationship of various hydrolytic enzymes, including PO, cellulase, phosphatidase and protease, with electrolyte leakage from healthy tissue. These apparent conflicts with Thatcher’s (1942) results can be resolved by Hancock’s (1972) findings that increases in permeability and electrolyte leakage does indeed occur in detached, senescing celery stalks as used by Thatcher but not in intact celery or sunflower in which permeability decreases. Decreased permeability probably has little impact on *Sclerotinia* spp. but can adversely affect the host tissue by restricting growth, predisposing it to injurious effects and adversely affecting resistance to pathogen invasion. In view of these findings, water soaking of tissues and accumulation of copious fluids around infection hyphae (Lumsden and Dow, 1973; Newton et al., 1973) needs reevaluation. Instead of resulting strictly from leakage from host cells (Newton et al., 1973; Thatcher, 1942), perhaps liquid accumulation is a result of the increased osmotic pressure of invaded tissue. Thatcher (1942) suggested that the fungus is responsible for a flow of water from the lower plant parts to its own locality. The greater osmotic pressure of the pathogen hyphae and solutes in the fluid surrounding the hyphae can result in osmotic flow of water from other

regions. This fluid can allow transport of nutrients from distances and act as a milieu for enzyme reactions and for the diffusion of oxalic acid into uninvaded tissues several cells distant.

14.4 Oxalic Acid in the Host-Pathogen Interaction

Numerous reports indicate participation of oxalic acid (OA) in the infection process. Evidence of such involvement includes recovery of OA from infected host tissues and the ability of OA and culture filtrates to induce disease symptoms (Marciano et al., 1983; Riou et al., 1991). The importance of oxalic acid (OA) in the infection process using *Arabidopsis thaliana* as a model for studying *S. sclerotiorum* pathogenesis has been demonstrated by Dickman and Mitra (1992). The first report of OA association with *Sclerotinia* infections was made by de Bary (1886). Infected carrot tissue showed a strong acidic reaction, and nonvolatile acids were implicated in the pH change. It was determined that 0.319 per cent of the carrot tissue was oxalate mostly as the calcium salt. Maxwell and Lumsden (1970) detected 1.1, 31.4 and 48.3 mg of oxalate per gram dry weight of tissue at zero, two and four days after inoculation of bean tissue with *S. sclerotiorum*. A toxic metabolite produced by *S. sclerotiorum* causing white rot of crucifers was identified as oxalic acid by Rai and Dhawan (1976b).

Oxalic acid (ethanedioic acid) is produced by all classes of fungi, representing considerable variation in fungal ecology and physiology. Various theories have been proposed as to the function oxalic acid may play in such diverse niches including roles in pathogenicity, competition between fungal species and control of environmental nutrients and toxin (Dutton and Evans, 1996). The mechanisms by which oxalic acid functions to aid in pathogenicity are centered on several proposed modes of action.

1. Early in pathogenesis, oxalic acid accumulates in infected tissues and increases in concentration as host colonization advances (Bateman and Beer, 1965; Bateman, 1964). As oxalic acid increases, the extracellular pH decreases to around 4–5. Because many CWDEs have optimum pH values below 5.0. The decrease in pH effectively enhances their activity (Bateman and Beer, 1965; Margo et al., 1984; Marciano et al., 1983; Maxwell and Lumsden, 1970).
2. By lowering pH to or below levels for optimum CWDE activity, oxalic acid may also contribute to an escape from acidic PG inhibition by plant defense PGIPs (Favaron et al., 2004).
3. Secretion of oxalic acid is concurrent with chelation of Ca^{2+} and pectic materials from cell walls three to five cell layers in advance of the fungal hyphae (Smith et al., 1986). Studies have shown that PG alone is not able to hydrolyse Ca^{2+} pectate in the middle lameliae, but works in synergy with oxalic acid. Oxalic acid chelates cell wall Ca^{2+} allowing polygalacturonase to hydrolyse the pectate thereby disrupting the integrity of the host cell wall (Bateman and Beer, 1965;

Kurian and Stelzig, 1979). Additionally, Ca^{2+} dependent plant defense responses may be compromised (Bateman and Beer, 1965).

4. Oxalic acid was shown to suppress the oxidative burst, an important early plant defense response in the unrelated species tobacco and soybean (Cessna et al., 2000).
5. Oxalic acid manipulates guard cell function by inducing stomatal opening and inhibiting abscisic acid induced stomatal closure, thus inducing foliar wilting during infection by *S. sclerotiorum* (Guimaraes and Stotz, 2004).
6. Oxalic acid inhibits the activities of plant produced polyphenol oxidase (Magro et al., 1984; Marciano et al., 1983).
7. By lowering the ambient environmental pH, oxalic acid may effect the transcriptional regulation of pH regulated genes necessary for the pathogenesis and developmental life cycle of *S. sclerotiorum*. e.g., the putative *S. sclerotiorum* transcription factor *pacl*, a homologue of other known fungal pH sensing transcription factors and a virulence factor for *S. sclerotiorum* was found to be auto regulated and accumulate transcripts in parallel with increasing ambient pH (Rollins and Dickman, 2001; Rollins, 2003). Likewise, *smk1*, a MAPK necessary for sclerotial development was maximally expressed under acidic pH conditions resulting from oxalic acid accumulation (Chen et al., 2004).
8. Oxalic acid can be indirectly toxic to the plant, most likely due to the acidic conditions resulting from oxalic acid production (Noyes and Hancock, 1981). Because oxalic acid production and endo PG activity are regulated by pH in *S. sclerotiorum* (Rollins and Dickman, 2001), the low environmental pH may weaken plants and make them more susceptible to subsequent fungal growth.

Culture medium pH has been shown to significantly influence oxalic acid production (Maxwell and Lumsden, 1970; Rollins and Dickman, 2001) via a self limiting mechanism, as oxalic acid accumulates, pH decreases and restricts further oxalic acid production. Although carbon source plays substantial role in the ability to synthesize oxalic acid, an alkaline environment increases oxalic acid biosynthesis independent of carbon source (Rollins and Dickman, 2001).

Oxalic acid production has been associated with pathogenesis of some *Sclerotinia* species (Bateman and Beer, 1965; Dutton and Evans, 1996; Godoy et al., 1990; Higgins, 1927; Kritzman et al., 1977; Magro et al., 1984; Marciano et al., 1983; Maxwell and Lumsden, 1970; Noyes and Hancock, 1981; Pierson and Rhodes, 1992; Stone and Armentrout, 1985). The evidence for such involvement is based on recovery of oxalic acid from infected tissues (de Bary, 1886; Ferrar and Walker, 1993; Godoy et al., 1990; Marciano et al., 1983), the correlation between these levels and disease severity (Bateman and Beer, 1965; Margo et al., 1984; Maxwell and Lumsden, 1970; Noyes and Hancock, 1981), and the development of *Sclerotinia* disease like symptoms after direct application of the toxin into plants (Bateman and Beer, 1965; Noyes and Hancock, 1981). Indeed, the secretion of oxalic acid by *S. sclerotiorum* results in the formation of lesions and water soaked tissue in advance of the invading fungal hyphae (Lumsden and Dow, 1973; Tu, 1985). Finally, mutants deficient in oxalic acid production, yet maintaining a full battery of CWDEs are non

pathogenic on dry bean whereas relevant strains that have regained their ability to produce oxalic acid display normal virulence patterns (Godoy et al., 1990). Similar results were obtained using *Arabidopsis thaliana* as the host plant (Dickman and Mitra, 1992). It can not be rules out, however, that the non pathogenicity of oxalic acid deficient mutants should partially be attributed to the reduced activity of CWDEs that rely on the lowering of environmental pH by oxalic acid *in planta*.

14.4.1 Role of Oxalic Acid in Host Tissues

The effect of OA in diseased tissues may be manifold. During the early stages of disease development and at advancing margins of lesions, OA may work synergistically with pectolytic enzymes as demonstrated for other similar diseases (Maxwell and Lumsden, 1970). Oxalic acid is a very strong chelator of calcium and other cations. In this capacity, OA would tie up mono- and divalent cations that inhibit maceration of tissue (Hancock, 1966) through inhibition of the action of endo and exo-PG. Oxalic acid *in vitro* stimulates the degradation of pectic substances by endo-PG, exo-PG and PME (Lumsden, 1979).

Oxalic acid also affects the pH of infected tissues. Changes in pH occur in infected tissues (Hancock, 1966; Lumsden, 1972, 1976; Maxwell and Lumsden, 1970; Morrall et al., 1972) and have been localized at the advancing margins of lesions (Lumsden, 1972). Values decrease from pH 5.0 to 4.0 at advancing margins of lesions on bean hypocotyls as determined by micro spectrophotometric methods (Lumsden, 1972). Increased acidity in the developing lesions favour the activity of endo- and exo-PG (Hancock, 1966; Lumsden, 1976), cellulase (Lumsden, 1969), hemicellulase (Hancock, 1967) and other hydrolytic enzymes (Lumsden, 1970), with pH optima for activity well below the pH of healthy host tissue and cell sap. Moreover, drastic pH changes have severe direct effects on cell viability and ability to respond to pathogen invasion. Increased acidity also favours rapid growth of the fungus (Maxwell and Lumsden, 1970).

Toxicity to host cells (de Bary, 1886; Hancock, 1972; Overell, 1952; Thatcher, 1942) resulting in death may be due to drastic pH changes or the cation chelation properties of GA. Oxalate at concentration and pH values detected in lesions (Maxwell and Lumsden, 1970) alone is sufficient to kill sunflower cells (Hancock, 1972). It has been noted, however, that a portion of the killing factor in lesions is heat labile (de Bary, 1886; Newton, 1972). There is possibility that pectolytic enzymes are involved in the killing action as with other diseases (Hancock, 1972). Overell (1952) discounted OA as a component of toxins secreted by *S. sclerotiorum* in plant tissues. This is based solely on the observation that OA is associated only with aging cultures. However, the pH, buffering capacity and glucose concentration in cultures of *Sclerotinia* spp. are very important in determining the production and the quantity of oxalate produced (Maxwell and Lumsden, 1970).

Oxalic acid also may be responsible for wilting symptoms usually associated with disease caused by *Sclerotinia* spp. (Pawlowski and Hawn, 1964; Noyes and

Hancock, 1981). Crystals identified as oxalate have been observed occluding xylem vessels (Lumsden and Dow, 1973). Vascular plugging (Pawlowski and Hawn, 1964; Noyes and Hancock, 1981) may be responsible for wilting or there may be a direct effect of oxalate on the water relationship in lamellar tissue. According to Noyes and Hancock (1981) during lesion development OA appears to enter the vascular stream and move systemically into the foliage. Oxalic acid apparently accumulates to a "critical" level in the leaves at which point wilt symptoms appear. Oxalic acid acts as a mobile toxin and causes the wilt syndrome.

Marciano et al. (1983) examined the relationship between cell wall degrading enzymes, OA and virulence in sunflower stems inoculated with two *S. sclerotiorum* isolates of different degrees of virulence. Oxalic acid does not directly affect cell wall degrading enzyme activity, although such activity is very sensitive to the variation in pH. Oxalic acid inhibits polyphenoloxidase (PPO), thereby limiting the production of the phenolic oxidation compounds that protect the pectic substances of cell walls to play a role in plant defense.

The importance of OA has been shown with mutants of *S. sclerotiorum* which do not synthesize OA (Godoy et al., 1990). The OA deficient mutants do not cause disease on *Phaseolus vulgaris* L. while the wild type and revertant mutants do cause disease. Further support for this is given by Favaron et al. (1988) who found that OA elicits production of the phytoalexin glyccollin-l in soybean hypocotyls. In contrast, in bean pods the secreted OA reduces the pH to a level where O-diphenol oxidase is inactive. It is postulated that this action enhances the pathogen's success by suppressing the host's defense mechanisms (Ferrar and Walker, 1993). Selection of alfalfa seedlings in four cvs for resistance to OA resulted in significant improvement in resistance to OA in each cv. but resistance to *S. trifoliorum* is improved in only one of the four cvs (Rowe and Welty, 1984). Callahan and Rowe (1991) used a host-pathogen interaction system (HPIS) which kept fungal mycelia from contacting a potential host to show that macromolecular components (>3,500 mol. wt.) and OA in exudates of *S. trifoliorum* are both important in inhibition of seedling growth in a bioassay. According to Rowe (1993), OA has a potential value in selection of resistance to either fungus in alfalfa or resistance to *S. sclerotiorum* in crimson clover (Fig. 14.4.1.1).

Oxalate production by *S. sclerotiorum* regulates guard cells during infection. Oxalate acts via accumulation of osmatically active molecules to induce stomatal opening and inhibition of ABA – induced stomal closer (Guimaraes and Stotz, 2004).

14.4.2 Response of Oxalic Acid in Tolerant and Susceptible Hosts

A bean tolerant cv. Ex Rico23 gets less severe disease symptoms and slower disease progress than susceptible cvs Kentwood, Seafarer and Fleetwood (Tu and Beversdorf, 1982). Later, Tu (1985) showed that tolerance of white bean to *S. sclerotiorum* is associated with tolerance to OA and that the difference in disease severity is paralleled by the rate of diffusion of OA solution in leaf tissue. Similarly,

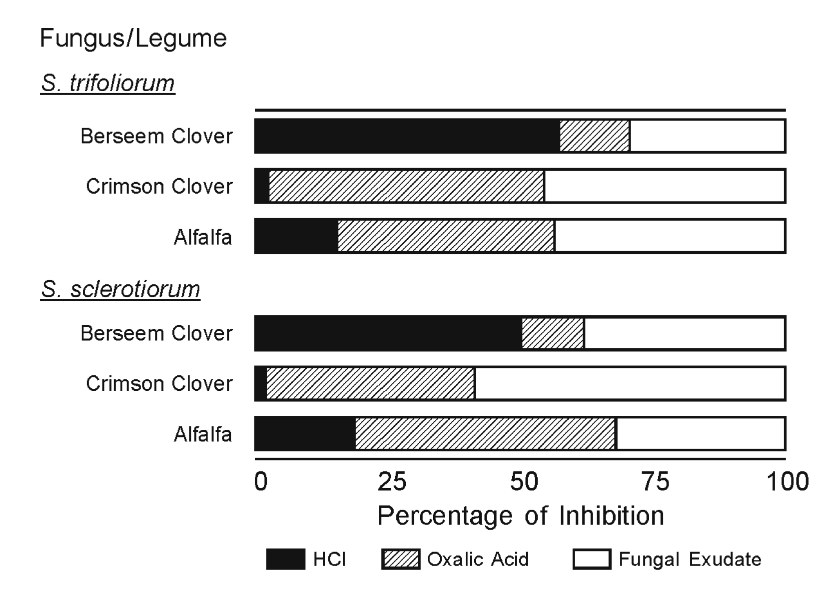


Fig. 14.4.1.1 Inhibition of seedling caused by oxalic acid and HCL expressed as cumulative proportions of the inhibition caused by fungal exudates of *Sclerotinia trifoliorum* and *S. sclerotiorum* on three forage legume species (Adapted from the publication of Rowe, 1993. With permission)

Noyes and Hancock (1981) observed that sunflower cvs resistant to *S. sclerotiorum* are more tolerant to OA than are susceptible cvs. Tariq and Jeferies (1985) showed that chloroplast degeneration in *Sclerotinia* infection is associated with OA secretion by the fungus. Such degeneration is apparently caused by the rupturing of chloroplast membranes by OA. It is possible that the accessibility of the chloroplasts to OA may be negatively correlated with the tolerance of the plasma membrane to OA. Thus, when plasma membranes of different tolerance are treated with a given concentration of OA, the membrane that is less tolerant loses stability more quickly than one that is more tolerant, loses selective permeability and allows free movement of OA into the cell. The different rates of membrane denaturization in a tolerant and susceptible cv. may explain why brown-rot lesions expand faster in leaves of susceptible plants than in those of tolerant ones during the pathogenesis of *S. sclerotiorum*. Tu (1989a) through electron microscopy noted cytological alterations induced by OA in tolerant and susceptible cvs. of beans to white mould. The plasma membrane of the resistant cv. appear more tolerant to damage induced by OA than that of susceptible cvs. At the same concentration of OA the plasma membrane and chloroplasts of the susceptible cv. are affected more and rupture more quickly, than those of the tolerant ones. In thin sections, the ruptured organelles are partially or completely disorganized. In replicas of freeze-fractured preparations of the plasma membrane, increased protrusions, wrinkles, breakage and ruptures are associated with increasing exposure to OA. Damage is more severe in the plasma membrane of the susceptible cv. than in that of the tolerant one. The susceptible cv. has higher conductivity than the resistant one.

Chapter 15
Disease Cycle

The life cycle of *Sclerotinia sclerotiorum* is a relatively simple one as compared to certain other fungi like rusts. The sclerotia are the perpetuating structures of *S. sclerotiorum*. Sclerotia may survive for three to five years in soil, assuring pathogen's availability when a host crop is planted. These survival structures become distributed throughout the tilled levels of soil. Although some sclerotia are destroyed by other organisms, a substantial number remain viable near the soil surface each year regardless of the host crop being grown (Adams and Ayers, 1979; Walker, 1969; Steadman, 1983; Willetts and Wong, 1980). When conditions are favourable, these sclerotia germinate to form either a mycelium or apothecia (Adams and Ayers, 1979; Purdy, 1958, 1979; Willetts and Wong, 1980). Mycelial production by sclerotia is negligible unless an exogenous source of energy is supplied (Abawi and Grogan, 1975; Willetts and Wong, 1980) and the infection of host plants can only occur if this energy source is available (Abawi and Grogan, 1975, 1979; Akai, 1981; Purdy, 1979). Mycelial infection occurs at or below the soil line (Abawi and Grogan, 1979) and has been reported in sunflower (Huang and Hoes, 1980) and in beans (Abawi and Grogan, 1975; Natti, 1971; Steadman, 1983).

In the case of white mould of beans, sclerotia ultimately germinate to produce ascospores (carpogenic germination) needed for infection to occur. The soil conditions that promote carpogenic germination are not well understood, however, sclerotia produced on an infected plant will not germinate to form apothecia until have been "preconditioned". This preconditioning, or physiological maturation, occurs during the winter or non cropped season. Freezing is not necessary. Sustained adequate moisture and cool temperatures (4–20°C) trigger the conversion of a dormant sclerotium into one that produces the sexual stage within a few weeks. Sclerotia must be at or within 5 cm of the soil surface for apothecial production to occur. When preconditioned sclerotia begin to germinate, stipes or apothecial stalks are formed. These may be formed in the light or under the soil without light, but light is necessary to stimulate formation of ascospore containing discs at the end of the stipes.

Since stipes are seldom longer than 5 cm, only sclerotia located within 5 cm of the soil surface complete spore production. Apothecia observed in the field are usually on the soil surface and are seldom raised above the soil surface by the stipes. This position on the soil and often under a plant is not the most advantageous for

G.S. Saharan, Naresh Mehta, *Sclerotinia Diseases of Crop Plants: Biology, Ecology and Disease Management*,

spore dispersal. When ascospores ripen or mature, however, a large number (10,000–30,000) mature simultaneously. Thus, when a sudden change in relative humidity triggers forcible discharge, many ascospores are released simultaneously, causing a "puffing" phenomenon that creates turbulence and assists aerial dispersal. Ascospores do escape above the canopy and have been detected on leaves 50–100 m from the source and in aerial samples collected above cropped fields. The importance of aerial dissemination in epidemics, however, has not been demonstrated. Sclerotia may be conditioned any time from fall harvest to bean flowering the next year, but in semiarid regions, carpogenic germination is usually initiated after the plant canopy has covered the soil surface. The requisite canopy development occurs in beans near the end of the first bloom and is responsible for a number of important micro meteorological events. In unshaded areas, soil temperatures near the surface could be above 30°C during the daytime and without frequent moisture from rain or irrigation, soil moisture would readily fall below field capacity. Neither the temperature nor the moisture situation would favour apothecial formation. Under the plant canopy, however, temperatures are seldom above 25°C and soil remains wetter between rains or irrigations. Optimum apothecial production occurs in 10 to 14 days at a soil matric potential ('I') of –0.25 bars (20% soil moisture in Tripp fine sandy loam soil) at a soil temperature of 15–18°C. In addition, the canopy tends to trap a large percentage of the more than two million spores produced by each apothecium during its five to ten days functional life. While somewhat limiting the potential for long-range spore dispersal, this tends to saturate available infection sites and promotes a high local infection potential (Steadman, 1983).

Mycelial germination and subsequent host infection is infrequent for sclerotia of *S. sclerotiorum* and is more often associated with small-sclerotial isolates of *Sclerotinia* spp., e.g., *S. minor* (Abawi and Grogan, 1979; Adams and Tate, 1976; Purdy, 1979; Willetts and Wong, 1980). It is even possible that many of the recorded occurrences where sclerotial mycelium is the primary source of inoculum are actually due to *S. minor* infection, not *Sclerotinia sclerotiorum,* even though *S. sclerotiorum* is cited as the disease-causing organism (Willetts and Wong, 1980). In most cases involving *S. sclerotiorum*, apothecia develop from sclerotia located either on the soil surface or buried in superficial layers of soil (Abawi and Grogan, 1979; Kruger, 1975a; Williams and Stelfox, 1980a). Large quantities of ascospores are forcibly discharged into the air (Abawi and Grogan, 1979; Purdy, 1979; Steadman, 1983; Walker, 1969; Willetts and Wong, 1980) and are carried by air currents for distances ranging from a few centimeters to several kilometers (Abawi and Grogan, 1979; Williams and Stelfox, 1979). However, according to Steadman (1983), honey bees efficiently distribute spores to the site of initial infection. Although functional aerial ascospores dispersal is somewhat limited in irrigated, semiarid regions. Spores as well as colonized plant debris and sclerotia can travel within and between bean growing areas in water moving through irrigation canals. In high rainfall areas, apothecial production in orchards or semi open areas can be more conducive to medium or long-range aerial dispersal. The introduction of sclerotia with seed or more rarely as infected seed probably plays a minor role in dissemination. The most important dispersal factor, however, is the long-term survival

of sclerotia associated with such a wide range of hosts. Outside semiarid regions, sclerotium germination may proceed crop planting. In New York, apothecia are formed in early spring just after snow melt and long before beans are planted. Similarly, in California and some southern locations, apothecia are produced during the late winter and early spring months (January, February and March), i.e., before beans have been planted. In fact beans and other crop hosts of *Sclerotinia* may be only incidental in its ecology, it can and often does survive on various weeds if crops are not available (Steadman, 1983).

Ascospores are responsible for most of the infections on the above-ground parts of susceptible plants (Abawi and Grogan, 1975, 1979; Willetts and Wong, 1980), including rapeseed (Dueck, 1977; Kruger, 1980; Morrall and Dueck, 1982; Williams and Stelfox, 1980a), beans (Abawi and Grogan, 1975, 1979; Steadman, 1983), tomato (Purdy and Bardin, 1953), sunflower (Huang and Hoes, 1980), cabbage, cauliflower, broccoli (McLean, 1958a) and many others. *S. sclerotiorum* is somewhat unique as a pathogen in that it requires an exogenous energy source for the ascospores to infect healthy or green plant leaves, pods, or stems. Senescent or injured organs on the plant or on the soil beneath the plant can provide the necessary exogenous energy. On beans, the most frequent source is the flower. After colonization of the flower, the fungal mycelium can infect adjacent green pods, leaves, or stems within two to three days. If ascospores are discharged before flowers or other senescent tissues are available, the spores can survive on plant surfaces or in the soil surface for nearly two weeks. Once a blossom is colonized, the mycelium remains viable for more than a month (Steadman, 1983).

When in contact with susceptible healthy host tissue, the ascosporic mycelium produces an appressorium (Abawi et al., 1975a; Lumsden, 1979; Purdy, 1958; Walker, 1969). Penetration occurs by the mechanical rupture of the host cuticle by means of a small hypha called an "infection hypha" or infection peg (Abawi et al., 1975; Lumsden, 1979; Walker, 1969; Willetts and Wong, 1980). Entry may also be gained through stomata, as reported for *S. trifoliorum* on clover (Prior and Owen, 1964; Walker, 1969) and for *S. sclerotiorum* on potato leaves (Lumsden, 1979). After entering the host plant, the fungus grows through the host tissues causing cells to die in advance of the invading hyphae (Grogan and Abawi, 1975; Maxwell and Lumsden, 1970; Purdy, 1958; Walker, 1969). Host tissues become disorganized by means of fungal enzymatic processes that affect the middle lamella between cells and cause cell wall breakdown (Lumsden, 1979; Morrall et al., 1972; Purdy, 1979; Walker, 1969; Willetts and Wong, 1980). The more succulent plant parts are invaded much more readily and rapidly than tissues in which the cell walls have become suberized or lignified (Walker, 1969).

Mycelium from colonized senescent tissue has the capacity to initiate infection, but mycelium from a sclerotium is unlikely to infect directly. The food reserve in a sclerotium apparently does not supply the energy necessary for formation of the infection cushions or appressoria and for subsequent entry into the host. Thus, even if sclerotia germinate to produce vegetative hyphal strands, it is much less likely that one or two sclerotia would be closer to senescent tissue than one or two million spores. Also, since sclerotia are soil borne, cannot be involved directly in initiating

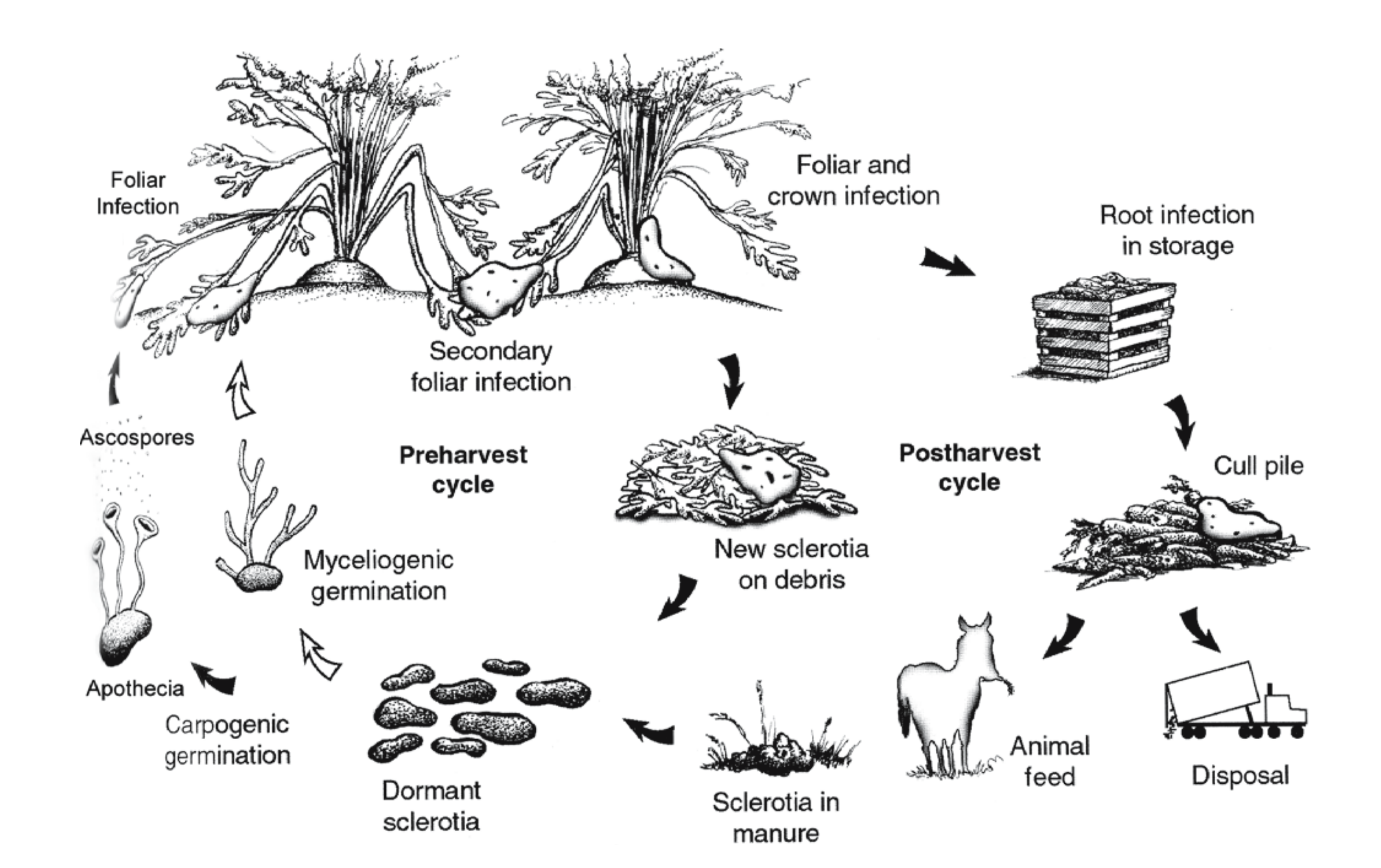


Fig. 15.1 Pre-harvest and post-harvest disease cycle of *Sclerotinia* rot of carrot caused by *Sclerotinia sclerotiorum* in a cropping system typical for temperate regions (Adapted from the publication of Kora et al., 2003. With permission)

infection in the above ground plant canopy (Steadman, 1983). The plant canopy influence is as great on spore germination, mycelial colonization and subsequent infection as it is on sclerotium germination. The ambient air temperature commonly reaches 40–45°C in semiarid regions in midsummer. The plant canopy temperature 10 cm above the ground, however, is only 25°C or less. Similarly, the dew point will be exceeded longer in the plant canopy than outside the canopy. Temperatures less than 30°C (the threshold temperature above which ascospores germination ceases) and plant surface moisture for 12–16 h recurring on a daily basis or continuous surface wetness for 42–72 h are prerequisite for white mould development, lower daytime temperature and lack of air movement that facilitates boundary layer exchange contribute to plant canopy microclimate differences (Steadman, 1983).

Secondary infection results from green tissue coming in contact with an infected area, but no secondary infection propagules are produced. Severe initial infections tend to be self-limiting and the dying plant no longer has the canopy to provide the necessary microclimate for further infection. Continuous germination of sclerotia and continuous spore discharge from each apothecium ensure adequate infection potential over a three to four week period. The limited hyphal growth emanating from newly produced sclerotia does not appear to play a role in secondary infection (Steadman, 1983).

After growing through the host tissues for several days, the mycelium produces sclerotia externally on affected plant parts and/or internally in stem pith cavities, fruit cavities, or between plant tissues (Dueck, 1977; Purdy, 1979; Purdy and Bardin, 1953). In field crops, sclerotia eventually reach the soil surface as they become dislodged by wind (Schwartz and Steadman, 1978) and/or as a result of harvesting and threshing operations where the sclerotia remain on the field with the crop debris (Grogan, 1979; Kruger, 1975b; Schwartz and Steadman, 1978). Some sclerotia are buried in the soil by subsequent tillage operations (Grogan, 1979; Kruger, 1975b; Schwartz and Steadman, 1978). The sclerotia survive in the soil and in plant debris to complete the disease cycle. In soybean, the fungus is reported to be internally seed-borne (Thompson and vander Westhuizen, 1979) but its role in the disease cycle has not been well understood. Apothecia are produced after sclerotia have been "conditioned" or mycelium may develop from sclerotia in soil, thus completing the cycle (Purdy, 1979).

In beans, primary infections are initiated chiefly by ascospores, which germinate with the help of free moisture and exogenous energy sources of fallen flower petals and necrotic tissues (Steadman, 1979). Other means of primary infection are associated with ascospore contaminated leaves in contact with moist soil and leaves coming in contact with sclerotia of the soil surface (Tu, 1989b). Secondary infections are achieved by natural contact of healthy plant parts with diseased ones. Later, many black resting bodies (sclerotia) of the fungus are formed on or in the infected stem tissues. The sclerotia are eventually incorporated into the soil with infected plant debris to survive for several years. Sclerotia buried at depths of less than 3 cm germinate to produce apothecia, each containing millions of ascospores (Tu, 1997).

The pre-harvest and post-harvest disease cycle of *Sclerotinia* rot of carrot by *S. sclerotiorum* in a cropping system typical for temperate regions is depicted in Fig. 15.1. (Kora et al., 2003).

Chapter 16
Epidemiology of *Sclerotinia* Diseases

Species of the genus *Sclerotinia* can function either as soil borne or airborne pathogens. Infections of above-ground plant parts result from ascosporic inoculum, whereas soil line infection may result either from ascospores or sclerotia. Below-ground infection, however, results from mycelial germination of soil borne sclerotia. Accordingly, the epidemiology of these two types of infections incited by *Sclerotinia* spp. is quite different and the effect of weather factors on their incidence and development differs considerably. Abawi and Grogan (1979) proposed that generally, the large sclerotia-type isolates of *Sclerotinia* (represented by *S. sclerotiorum* and *S. trifoliorum*) function primarily by producing apothecia and that mycelial germination from sclerotia contributes minimally, if at all, to the development of epidemics. In contrast, infections incited by the small sclerotial type isolates (*S. minor*) originate primarily through the eruptive mycelial germination of sclerotia, whereas production of apothecia under natural conditions occurs very rarely and thus is of minor importance. The different modes of infection exhibited by the large- and small sclerotia type isolates of *Sclerotinia* probably results from continued adaptation of these species to their ecological niches. Only a limited amount of detailed epidemiological information is available on a few important diseases. Furthermore, quantitative epidemiology data are essentially lacking (Abawi and Grogan, 1979). The information generated so far on epidemiology of different host-pathosystem is as follows.

16.1 White Mold of Beans

Most infections of beans occur on above ground plant parts and it seems unlikely that the large sclerotia per se of *S. sclerotiorum* are an important form of inoculum that function essentially to produce airborne ascospores (Abawi and Grogan, 1975; Cook et al., 1975; Saito, 1977; Schwartz and Steadman, 1978; Suzui and Kobayashi, 1972a, b, c). The incidence of disease in different fields ranges from a trace to 100 per cent (Tu, 1989b).

G.S. Saharan, Naresh Mehta, *Sclerotinia Diseases of Crop Plants: Biology, Ecology and Disease Management*,

16.1.1 Source of Inoculum

White mould of beans has been listed as a seed borne disease by Nobel and Richardson (1968). The transmission of the fungus by or with bean and other types of seed has been reported (Baker and Davis, 1951; Blodgett, 1946; Hungerford and Pitts, 1953; Nicholson et al., 1972). According to Akai (1981) and Steadman (1975), *S. sclerotiorum* can be disseminated in seed, but this is unlikely to be of epidemiological significance. However, Tu (1988) reported that in dry bean internally infected seeds are important to the spread of the disease by producing sclerotia in the soil after the seeds are planted. White mould epidemics of beans are initiated by ascospores produced by sclerotia of *S. sclerotiorum* (Abawi and Grogan, 1975; Abawi et al., 1975a; Cook et al., 1975; Saito, 1977; Schwartz and Steadman, 1978; Suzui and Kobayashi, 1972a, b). Only sclerotia in the top 2–3 cm of the soil are functional because apothecia with stipes longer than 3 cm rarely are produced under field conditions. Sclerotia present in and outside bean fields also can provide ascosporic inoculum for bean white mould epidemics. In New York, ascosporic inoculum originates mainly from sclerotia outside of bean fields (Abawi and Grogan, 1975). Sclerotia often are found producing apothecia around the base of dandelion plants and wild clover, or near other host plants in hedge rows, uncultivated wooded areas and fruit orchards. In drier areas such as Nebraska and California, apothecia are produced in bean fields that are sprinkler or furrow irrigated (Cook et al., 1975; Schwartz and Steadman, 1978). Under these conditions, water required for sclerotial germination is provided by irrigation, but apothecial production is nil prior to the development of a dense canopy of plants over the soil that decreases evaporation of water from the soil surface. As a consequence, low moisture tensions requisite for sclerotial germination are maintained long enough for apothecial production. In such areas, sclerotia may be distributed within and between fields in irrigation water (Schwartz and Steadman, 1978). Mycelium from sclerotia has been reported to infect beans (Natti, 1971). However, Abawi and Grogan (1975) suggested that this occurs rarely under natural conditions. Abawi and Grogan (1979) observed that sclerotia placed in direct contact with bean tissues fail to infect, even after prolonged incubation under near optimum conditions because a non-living food base is not available, infection does not occur. Instead of infecting directly by production of mycelium, the sclerotia produced an average of more than three apothecia per sclerotium. Preconditioned (functionally mature) sclerotia of *S. sclerotiorum* usually produce very sparse mycelial growth when utilizing their own reserve energy. Such mycelium can infect beans only when a readily available energy source is present in direct contact between the sclerotium and bean tissues.

16.1.2 Dissemination of Inoculum

Sclerotinia sclerotiorum spreads from field to field and from one geographical area to another by several means. Field to field spread depends primarily on windblown

ascospores during the growing season (Muckel and Steadman, 1981) and secondarily on sclerotia in contaminated soil, irrigation water and manure spread on fields from animals fed with infected plant materials (Adams and Ayers, 1979). However, Adams and Ayers (1979) and Tu (1988) suggested that the greatest potential for long distance dissemination of *Sclerotinia* spp. might be by seeds infected with mycelium or contaminated with sclerotia.

Generally, apothecia are produced and ascospores of *S. sclerotiorum* are discharged throughout the growing season if the moisture of the top 2–3 cm of soil is maintained near saturation. Under New York conditions, mature apothecia have been found as early as 20 April, provided that the snow has melted and soil temperature has reached 10°C for at-least part of the day. When moisture is not a limiting factor, apothecia are produced throughout the bean growing season in New York which begins with planting in the first week of May and continues until harvest in mid September (Abawi and Grogan, 1979). Only limited information is available concerning the liberation, transport and deposition of ascospores of *S. sclerotiorum* (Suzui and Kobayashi, 1972b). It seems that ascospore liberation and transport in *Sclerotinia* spp. closely resembles that reported for other discomycotina (Hirst, 1959; Ingold, 1960). Each day, when subjected to a slight decrease in moisture tension, the mature asci forcibly discharge ascospores into the air to a distance of more than 1 cm. This height of discharge enables the ascospores to escape the still layer of air near the soil surface and to reach the more turbulent above ground layers. It has been reported that the longest dispersal of ascospores of *S. sclerotiorum* is 25 m (Suzui and Kobayashi, 1972b) and several kilometers (Brown and Butler, 1936). Dispersal to the latter distances is possible and probably not uncommon (Abawi and Grogan, 1979). It has been estimated that ascospore production by a single apothecium may be as high as 3×10^7 ascospores and a single sclerotium may produce 2.3×10^8 ascospores (Schwartz and Steadman, 1978).

A mucilaginous material is discharged along with ascospores that can cement the spores to host tissues or other objects encountered during light. Ascospores deposited on bean tissues need not infect immediately, but can survive for a considerable time until the wet conditions and exogenous energy sources required for infection become available (Grogan and Abawi, 1975). Under laboratory conditions, the thin walled ascospores of *S. sclerotiorum* survive for 21 days at 7 per cent RH, but survival is less than 5 days at 100 per cent RH. Ascospores atomized onto bean leaves under field conditions survive for as long as 12 days. Ascospore mortality on the topmost leaves of beans during 48 h period is significantly positively correlated with the number of hours at or above 21.1°C during the same period. Ascospore survival on shaded leaves ≥12–15 cm above ground within dense canopy averages 21.5 per cent greater than on the topmost leaves. Few ascospores survive on the topmost leaves after 6 days in the field (Figs. 16.1.2.1–16.1.2.8), with ascospore viability ranging from 7 to 0 per cent after 6 and 14 days respectively (Caesar and Pearson, 1983).

% ASCOSPORE GERMINATION

5 C: 10%, 34%, 59%, 80%, 98%, 100%

20 C: 10%, 34%, 59%, 80%, 98%, 100%

10 C: 10%, 59%, 34%, 80%, 98%, 100%

25 C: 10%, 34%, 59%, 80%, 98%, 100%

15 C: 10%, 34%, 59%, 80%, 98%, 100%

30 C: 10%, 34%, 80%, 60, 98%, 100%

DAYS

Fig. 16.1.2.1 Effect of temperature and relative humidity on the survival of ascospores of *S. sclerotiorum* ejected onto glass cover slips and held over saturated salt solutions with different equilibrium humidities. Each line represents one relative humidity treatment (Adapted from the publication of Caesar and Pearson, 1983. With permission)

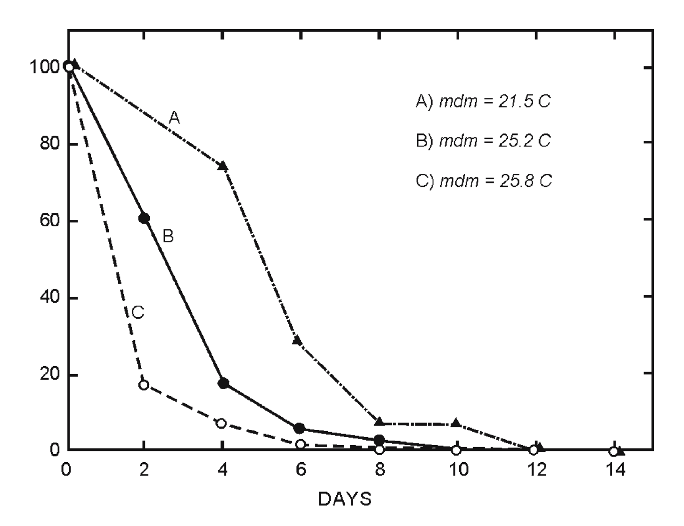


Fig. 16.1.2.2 Survival of ascospores of *S. sclerotiorum* on the topmost bean leaves in the field under three temperature regimes (Adapted from the publication of Caesar and Pearson, 1983. With permission)

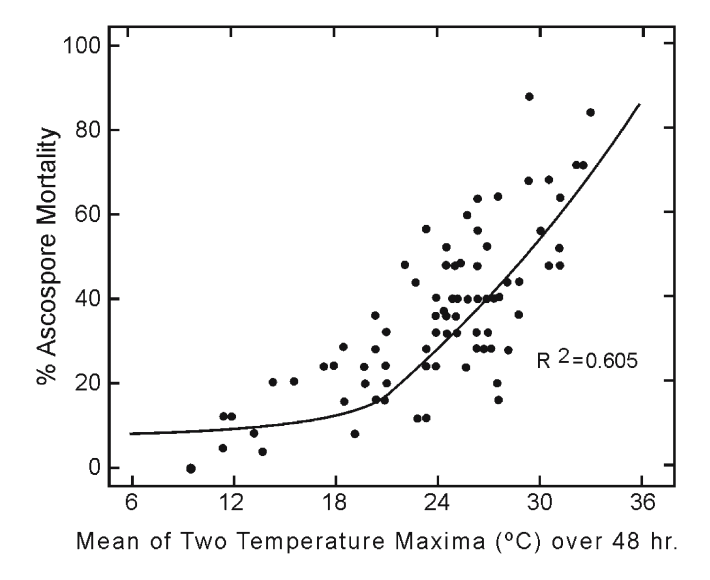


Fig. 16.1.2.3 Mortality of ascospores of *S. sclerotiorum* in the field on the topmost bean leaves (Adapted from the publication of Caesar and Pearson, 1983. With permission)

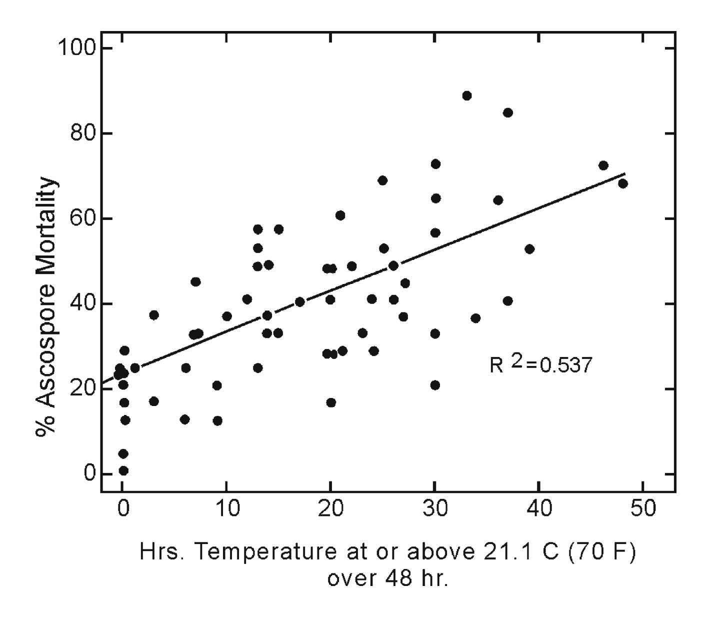


Fig. 16.1.2.4 Mortality of ascospores of *S. sclerotiorum* in the field on the topmost bean leaves (Adapted from the publication of Caesar and Pearson, 1983. With permission)

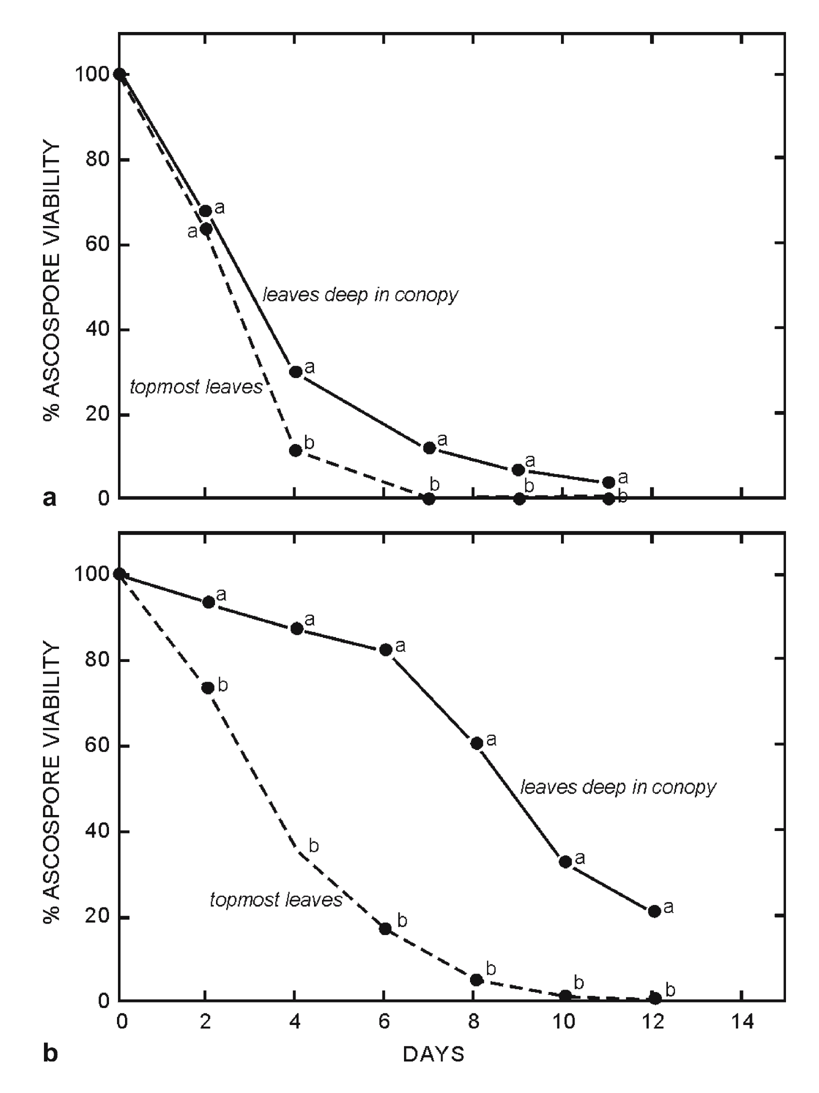


Fig. 16.1.2.5 Survival of ascospores of *S. sclerotiorum* on bean leaves at the top of the plant canopy and leaves deep in the canopy. (A) Mean daily maximum temperature 29.9°C; (B) Mean daily maximum temperature 24.3°C (Adapted from the publication of Caesar and Pearson, 1983. With permission)

Fig. 16.1.2.7 Effect of solar radiation on survival of ascospores of *S. sclerotiorum* in the field under various plastic films with different ultraviolet transmission properties. (A) Ascospores on topmost leaves of bean plants unsheltered or sheltered with type A Mylar; (B) ascospores on

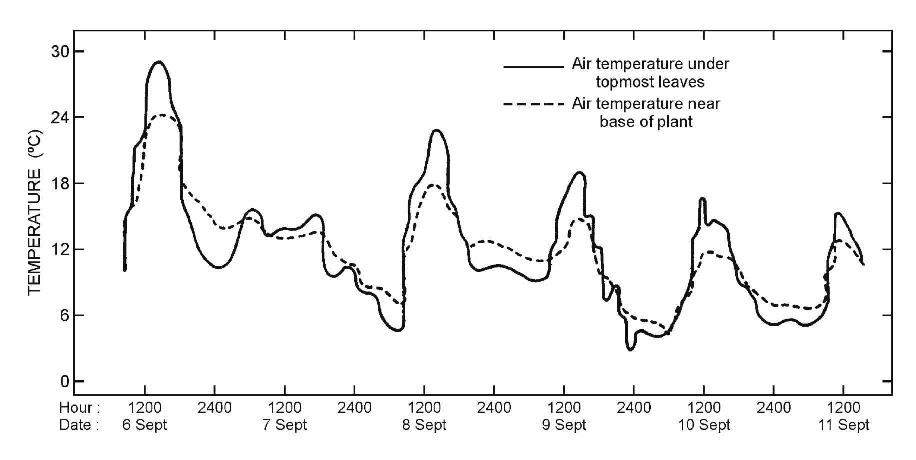
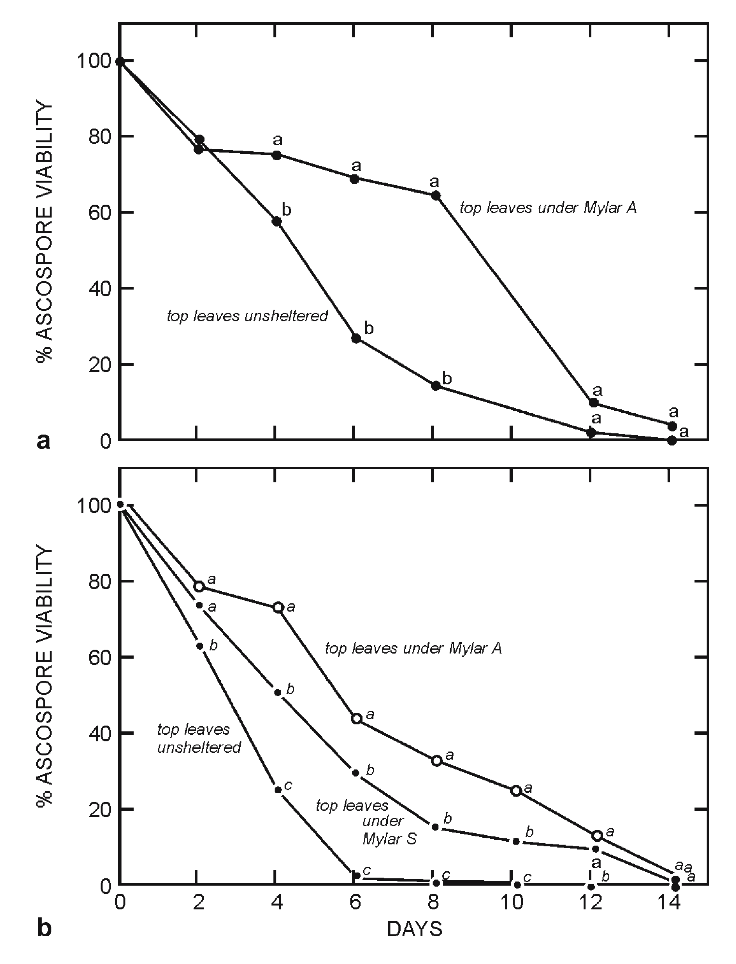


Fig. 16.1.2.6 Recording of air temperature under the topmost leaves and at the base of the plant in a dense bean canopy (Adapted from the publication of Caesar and Pearson, 1983. With permission)



topmost leaves of bean plants unsheltered or sheltered with type S Mylar or type A Mylar (Adapted from the publication of Caesar and Pearson, 1983. With permission)

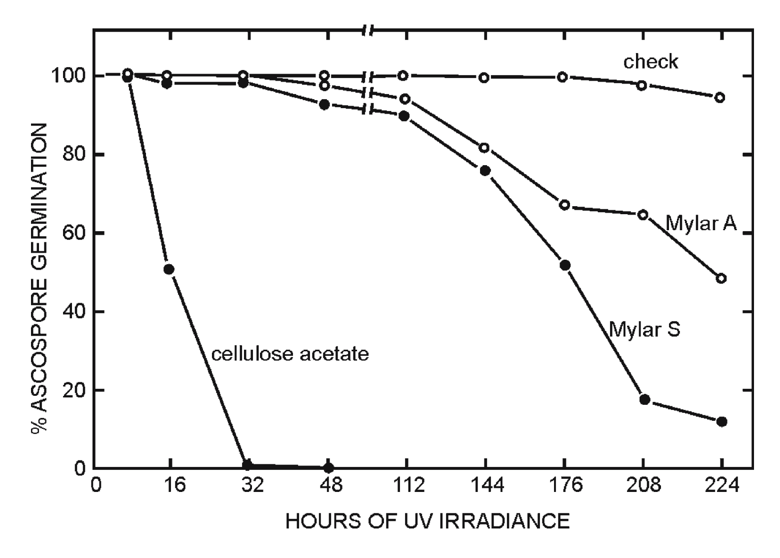


Fig. 16.1.2.8 Survival of ascospores of *S. sclerotiorum* after exposure to ultraviolet (UV) radiation (3.2×10^5 J/m^2 estimated dosage per 32 h exposure period at 250–320 nm) from two FS-40 sunlamp fluorescent tubes differentially filtered with three plastic films; 0.27-mm cellulose acetate; 0.0254-mm type S Mylar and 0.127-mm type A Mylar (Adapted from the publication of Caesar and Pearson, 1983. With permission)

16.1.3 Factors Affecting Production of Ascosporic Inoculum

Only preconditioned and functionally mature sclerotia are capable of producing ascosporic inoculum for white mould epidemics. The time required for preconditioning sclerotia varies for different isolates, however, optimum conditions required for preconditioning the sclerotia have not been determined precisely. In general, however, newly formed sclerotia require some time under cool moist conditions before attaining the capability for carpogenic germination. Several factors are known to influence carpogenic germination of preconditioned sclerotia of *S. sclerotiorum* (Coley-Smith and Cooke, 1971). However, prolonged high soil moisture is the most common limiting factor (Abawi and Grogan, 1975; Coley-Smith and Cooke, 1971; Grogan and Abawi, 1975). Continuous moisture for about ten days is required for apothecial development, and even a slight moisture tension (osmotic or matric or a combination of both) prevents apothecial formation. Field collected sclerotia capable of carpogenic germination when sampled earlier, after exposure to extreme drying conditions on the soil surface of fields in New York, failed to produce apothecia when placed under near-ideal conditions in a growth chamber for as long as three months. Thus, exposure to extreme drying and possibly high temperature has a prolonged detrimental effect on apothecial production, however, the sclerotia remain viable as indicated by consistent mycelial production on nutrient media. Duniway et al. (1977) reported that the optimum water potential for apothecium production of bean isolates from New York and Nebraska is −80

to −160 mb and −240 mb, respectively. This difference may reflect the relative adaptation of the isolates of *S. sclerotiorum* from Nebraska to reduced soil moisture tension. It also indicates that maintaining soil water at near field capacity (−300 mb) for long periods of time is essential for carpogenic germination. Soil water content, especially in the top 2–3 cm of soil, varies considerably and is affected by weather parameters such as RH, wind velocity, type and extent of plant canopy and temperature. Temperature also exerts a significant effect on apothecial production by *S. sclerotiorum* (Abawi and Grogan, 1975; Coley-Smith and Cooke, 1971; Saito, 1977). Preconditioned sclerotia incubated in water at different constant temperatures produces the most initials and mature apothecia at 11°C. However, production at 15°C also is quite good. No apothecial initials are produced at either 30°C or 5°C after 21 days of incubation (Abawi and Grogan, 1979).

16.1.4 Factors Affecting Host Infection and Disease Development

In general epidemics of white mould of beans occur only after flowering. However, a few infected plants can be observed occasionally in fields prior to blossoming. Ascospores of *S. sclerotiorum* require an exogenous energy source to infect healthy bean plants (Abawi and Grogan, 1975; Abawi et al., 1975b; Cook et al., 1975; Purdy, 1958). Under field conditions, mature bean blossoms usually serve as an energy source. However, ascospores readily infect mechanically injured plants and also nonflowering beans with genetically induced necrosis or with necrotic lesions incited by other plant pathogens (Abawi et al., 1975b). This may explain the occasional occurrence of white mould prior to blossoming. Ascospores completely colonize mature and senescent blossoms within two to three days (Abawi et al., 1975b) and mycelial growth from these colonized blossoms produces infection by contact with leaf, stem and pod tissues (Akai, 1981). Numerous reports have stressed the importance of moisture in the development of white mould of beans (Abawi and Grogan, 1975; Cook et al., 1975; Grogan and Abawi, 1975; Moore, 1955; Natti, 1971; Schwartz and Steadman, 1978). Infection of beans by *S. sclerotiorum* occurs only if free moisture is maintained for a relatively long period at the interface of bean tissues and the inoculum (Abawi and Grogan, 1975). Approximately 48–72 h of continuous leaf wetness are required for infection by ascospores. Similarly, 16–24 h and over 72 h of leaf wetness are required for the infection of beans by moist, infected bean blossoms with actively growing mycelium and dry, colonized bean blossoms, respectively. Furthermore, high RH, even near 100 per cent is not sufficient for lesion initiation. Infection of detached leaves enclosed in plastic boxes with free water in the bottom usually fails unless the leaves are sprayed periodically with water. In addition, expansion of lesions also requires free moisture. Lesion enlargement is stopped abruptly if the surface of infected tissues (except bulky stem tissues) becomes dry. However, the dry lesions can resume expansion when free water becomes available. The duration of leaf wetness and frequency of rainfall or irrigation when inoculum is available is more important than the total amount of

water (rainfall or irrigation) received (Abawi and Grogan, 1975; Schwartz and Steadman, 1978). The disease is more prevalent in fields with heavy vegetative growth and in the areas where air circulation is limited, such as low lying fields and particularly those surrounded by uncultivated wooded areas (Haas and Bolwyn, 1972; Natti, 1971). Disease incidence is about 13 times greater in the heavily irrigated plots than in the normally irrigated plots (Weiss et al., 1980a, b).

Ascospore germination, its growth as well as lesion initiation and development are near optimum at constant temperatures of 10–25°C as depicted in Fig. 16.1.4.1 (Abawi and Grogan, 1975; Suzui and Kobayashi, 1972a; Weiss et al., 1980b). Furthermore, lesions do not develop on inoculated leaves of plants incubated at 5°C or 30°C. In the irrigated and hotter regions where beans are grown, higher temperatures may in some instances limit white mould incidence and development (Abawi and Grogan, 1979). However, in the heavy irrigation plots air, leaf and soil temperatures are consistently lower which lead to severe disease (Figs. 16.1.4.2, 16.1.4.3) in cv. GH Tara (Weiss et al., 1980b).

Under moist conditions, leaf, stem and pod tissues in contact with infected blossom parts develop water soaked lesions. These lesions continue to enlarge and within a few days covered with a dense white mycelial mat. Usually, numerous sclerotia are produced on the surface of the mycelium within seven to ten days. The fungus continues to grow and may invade the whole plant above the ground. Under a dense canopy of foliage, fallen leaves, blossoms and other plant parts become infected, with the result that mycelium grows over the soil surface. Since asexual spores are not produced by *S. sclerotiorum*, plant to plant infection occurs only through direct hyphal growth from previously infected tissues. Newly produced sclerotia are capable only of limited hyphal growth unless provided with an exogenous food base and usually exhibit dormancy for carpogenic germination (Abawi and Grogan, 1975; Coley-Smith and Cooke, 1971; Cook et al., 1975; Saito, 1977).

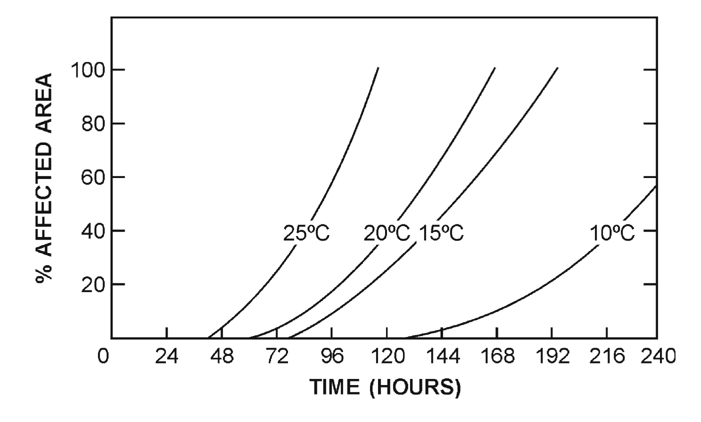


Fig. 16.1.4.1 Percentage of leaf area affected by white mold (*S. sclerotiorum*) of dry edible bean plants as a function of time after inoculation and temperature (Adapted from the publication of Weiss et al., 1980b. With permission)

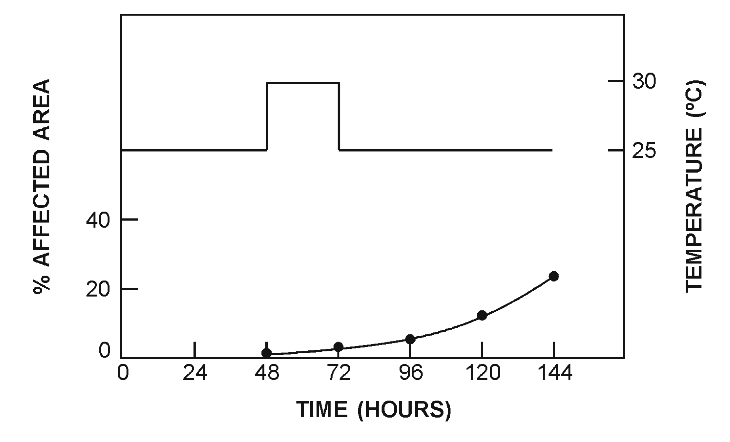


Fig. 16.1.4.2 Influence of a step change in temperature of limited duration on percentage of leaf area affected by white mold (*S. sclerotiorum*) of dry edible bean plants (Adapted from the publication of Weiss et al., 1980. With permission)

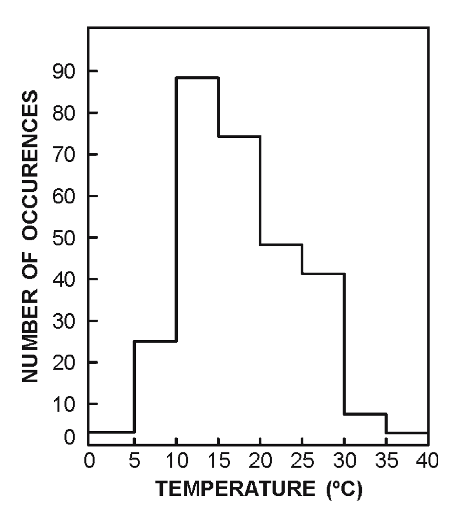


Fig. 16.1.4.3 Distribution of hourly average air temperatures (in 5°C intervals) at 10 cm above ground in Great Northern cultivar (Adapted from the publication of Weiss et al., 1980. With permission)

Secondary spread of *S. sclerotiorum* on snap beans occurs only to a limited extent and it is believed that it plays a minor role in the development of epidemics. However, secondary spread by plant to plant mycelial growth may be more important on dry beans as a result of the longer period of susceptibility due to indeterminate flowering and the moist chamber effect produced by the dense canopy of foliage. White mould appears to be a simple interest disease (Abawi and Grogan, 1979).

At least four types of primary infection has been shown in navy bean (*Phaseolus vulgaris*) by *S. sclerotiorum*: (1) First infection resulting from ascospores on contaminated flower petals which lodge on the stem; (2) Infection associated with ascospore contaminated leaves in contact with moist soil; (3) Infection associated

with injuries and necrotic tissues and (4) Leaves coming in contact with sclerotia on the soil surface. Ascospore contaminated or infected tissues need not necessarily result in a disease lesion. The progression of disease may be impeded if high relative moisture is not continuously available. These observations suggest that infection not only occurs from ascospores colonizing senescent tissues, but also by myceliogenic germination of sclerotia (Tu, 1989b).

The epidemiology of white mould of beans is dependent on several factors such as: soil inoculum, soil moisture, rainfall, cultivar susceptibility, row width and plant density. Fields with high inoculum density and high soil moisture have high disease incidence because disease initiation is favoured by cool and damp soil conditions. Secondary spread occurs through plant to plant contact. The ratio of within row spread to between row spread is approximately 6, 4, 3 and 1.5 for row widths of 80, 60, 40 and 20 cm, respectively. Disease incidence increases with reduction in row width and increase in plant density. Cultivar susceptibility is an important factor in disease epidemiology. In a susceptible cv. (Fleetwood), the percentage of infected plants increases from 0 to 100 per cent in four week in 80 cm row planting while in tolerant cv. (Ex Rico 23), it progresses from 0 to 35 per cent. The frequency of rainfall is a more important factor in disease epidemiology than total rainfall (Tu, 1989a, 1997).

16.2 Lettuce Drop

Lettuce drop can be incited by either *S. sclerotiorum* or *S. minor*. These two species may occur together in the same field. However, *S. minor* usually is the predominant species on lettuce (Adams and Tate, 1975; Jarvis and Hawthorne, 1972; Marcum et al., 1977; Newton and Sequeira, 1972b). Infection of lettuce with *S. sclerotiorum* almost always occurs at the ground level because it usually originates from ascosporic infection of senescent lower leaves. In contrast, infection with *S. minor* can occur either at the soil line through senescening lower leaves or below ground as deep as 10 cm through root and stem tissues. Production of apothecia, by at least some isolates of *S. minor* is known, but their natural occurrence is not that common (Beach, 1921; Hawthorne, 1976; Jagger, 1920). Thus, the role of ascospores in lettuce drop epidemics caused by *S. minor* appears to be of minor importance. If involved, ascospores of *S. minor* probably can infect senescent lower leaves as does *S. sclerotiorum* (Abawi and Grogan, 1979). Usually, however, the occurrence of *S. sclerotiorum* is more sporadic, it may not be active one year and yet may produce a widespread epidemic during the following year. In contrast, *S. minor* occurs more consistently year after year in infested fields. The epidemiology of lettuce drop caused by *S. sclerotiorum* is similar to that of white mould of beans. Development of both diseases is influenced by factors involved with production and infection by ascospores. Thus, this section is concerned primarily with the epidemiology of lettuce drop caused by *S. minor* and the comparison of it with *S. sclerotiorum*. Ascospores of *S. sclerotiorum* when inoculated in lettuce, germinate on leaves after

2–4 h of continuous leaf wetness at optimum temperature of 15–20°C after seven to nine days of inoculation with maximum level of 96 per cent (Young et al., 2004).

Drop of lettuce in Massachusetts caused by a small sclerotia type of *Sclerotinia* was described by Smith in 1900 (Smith, 1900). Because of failure to produce apothecia, it is concluded that the fungus is a "degenerative" form of *S. libertiana* that has become highly specialized as a vegetative parasite, able to renew growth in a vegetative manner by the direct production of mycelium. Later reports also indicated that the small sclerotia, when first formed, make no growth (remain dormant), but after a period of rest and dryness, send out mould like growth which attack plants. Its method of reproduction and spreading is strictly limited to soil. Stone and Smith (1900) reported that covering the surface of soil with a few inches of sterilized soil, completely controls drop, but drying of infested soil during August, September and October results in a marked increase in the incidence of drop in the next crop. Beach (1921) reported infection of lettuce heads that results from sclerotia buried in natural soil which suggests that a food base is not required. Beach (1921) observed drop caused by both *S. minor* and *S. sclerotiorum* and noted the following differences in their characteristics and behaviour:

(a) *Sclerotinia minor* produces smaller, but many more sclerotia than does *S. sclerotiorum.*
(b) Cultivation or other conditions that reduce the duration of soil dampness prevent the production of apothecia by *S. sclerotiorum,* but do not prevent the vegetative growth of *S. minor* thus, drop caused by *S. minor* can occur after a rainy period of too short duration for production of apothecia by *S. sclerotiorum.*
(c) *S. minor* tends to be more localized in distribution and spreads slower, but reoccurs more consistently year after year in infested fields. In contrast, *S. sclerotiorum* may occur with wide distribution in fields where very little or none had occurred in the previous year; the occurrence of *S. sclerotiorum* is associated with prolonged wet weather that is favourable for production of apothecia.
(d) Incidence of drop caused by *S. minor* or *S. sclerotiorum* generally increases if infected soils become dry prior to planting. Later studies concerning the comparative epidemiology of lettuce drop caused by *S. minor* or *S. sclerotiorum* generally is in agreement with Beach's conclusions that were published in 1918 (Adams, 1975; Adams and Tate, 1975, 1976; Hawthorne, 1974, 1976; Jarvis and Hawthorne, 1972; Marcum et al., 1977).

16.2.1 Source of Inoculum

Two types of asexual (vegetative) germination, hyphal or mycelial have been described for sclerotia of *S. minor* (Adams and Tate, 1976). Hyphal germination is characterized by the production of a few short hyphal strands that grow very little without an exogenous food base. Hyphal germination of the large sclerotial isolates of *S. sclerotiorum* is similar. This type of inoculum will infect lettuce only through

prior colonization of exogenous energy sources (Adams and Tate, 1976). In contrast, eruptive mycelial germination of sclerotia appears first as bulges in the sclerotial rind which eventually ruptures and exposes a massive, dense mycelium that utilizes stored food reserves in the sclerotium for growth as does *S. sclerotiorum* during carpogenic germination. The sclerotia of *S. minor* have a dormancy period prior to mycelial germination and the length of this dormancy varies among isolates and is affected by media composition, time after formation, drying and other undetermined factors. Sclerotia that undergo mycelial germination are capable of infecting lettuce directly without the need for an exogenous food source, thus the infective propagules for lettuce drop caused by *S. minor* is the sclerotium per se instead of ascospores as it is with *S. sclerotiorum*. Production of apothecia by sclerotia of *S. minor* has been reported and the role of ascospores in initiation of lettuce drop caused by *S. minor* has been suggested (Beach, 1921; Hawthorne, 1976; Jagger, 1920; Jarvis and Hawthorne, 1972).

An inoculum density of two to seven sclerotia per 100 mg of soil causes about 10 per cent lettuce drop. The incidence of lettuce drop is about 20 and 80 per cent at inoculum densities of 31 and 250 sclerotia per 100 g of soil, respectively. The sclerotial populations of lettuce field soils in New Jersey vary from 0 to 20 and average 3.9 sclerotia per 100 g of soil (Abawi and Grogan, 1979). Inoculum densities of *S. minor* in lettuce fields in Salinas Valley, (California) range from zero to three sclerotia per 70 ml (100 g) sample of soil. Sclerotia capable of eruptive germination and thus potentially able to cause infection (competent) range from 0 to 0.80 per 70 ml of soil. Incidence of drop ranges from 0.5 to 18.5 per cent and is proportional to both inoculum density of competent sclerotia ($r = 0.80$) and ($r^2 = 0.76$) total sclerotia (Imolehin and Grogan, 1980). However, according to Dillard and Grogan, (1985), inoculum densities at lettuce planting time range from 1.66 to 11.35 sclerotia of *S. minor* per 100 cm^2 of soil. The spatial pattern of sclerotia within plots is best described by the negative binomial distribution (Figs. 16.2.1.1–16.2.1.4) and the propagules are mostly clumped or clustered in the field (Tables 16.2.1.1, 16.2.1.2).

Dynamics of lettuce drop incidence and *S. minor* inoculum under varied crop rotations determined by Hao and Subbarao (2006) indicated that in 100 cm^3 of soil, a minimum of 4–5 sclerotia are needed for 100 per cent infection. The degree of aggregation of sclerotia (*S. minor*) and lettuce drop incidence increases significantly during cropping season under furrow irrigation (Fig. 16.2.1.5), but not under subsurface drop irrigation (Wu and Subbarao, 2003).

16.2.2 Dissemination of Inoculum

Inoculum of lettuce drop is sedentary and spread between fields is slow and restricted. Below ground infection of roots and ground level infection of senescent leaves generally occurs when leaves and roots are in direct contact or are only a few millimeters away from germinating sclerotia. It has been suggested that hyphal webs of *S. minor* and infected debris may become airborne and spread within and

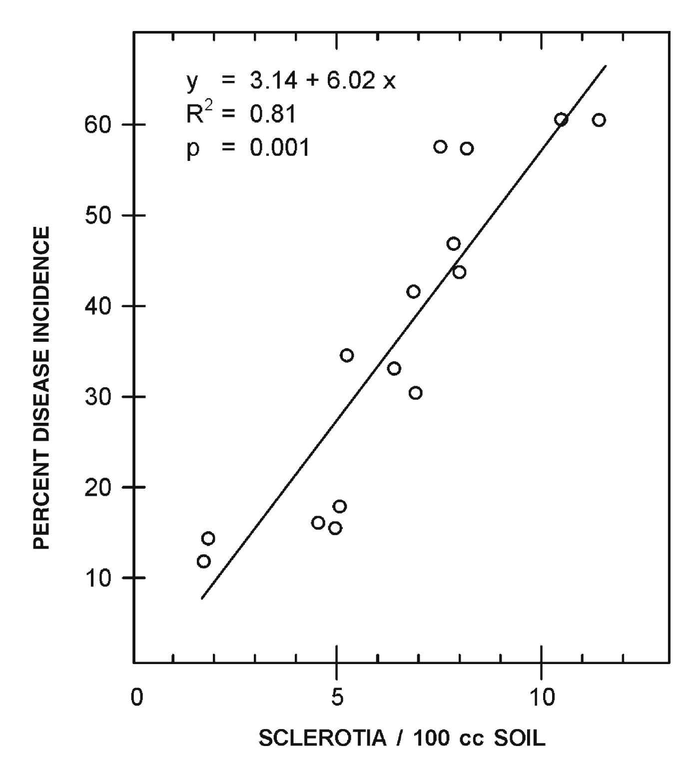


Fig. 16.2.1.1 Relationship between initial mean inoculum density of sclerotia of *S. minor* in 15 field plots at planting and disease incidence of lettuce drop at harvest (Adapted from the publication of Dillard and Grogan, 1985. With permission)

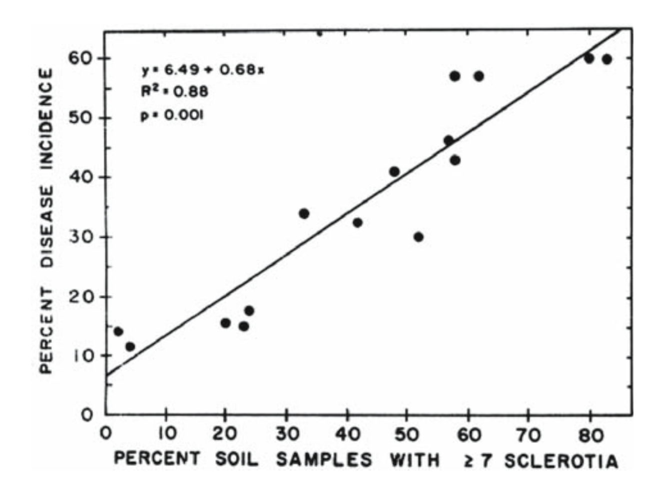


Fig. 16.2.1.2 Relationship between the percentage of soil samples with seven or more sclerotia of *Sclerotinia minor* at planting from 15 fields plots and disease incidence of lettuce drop at harvest (Adapted from the publication of Dillard and Grogan, 1985. With permission)

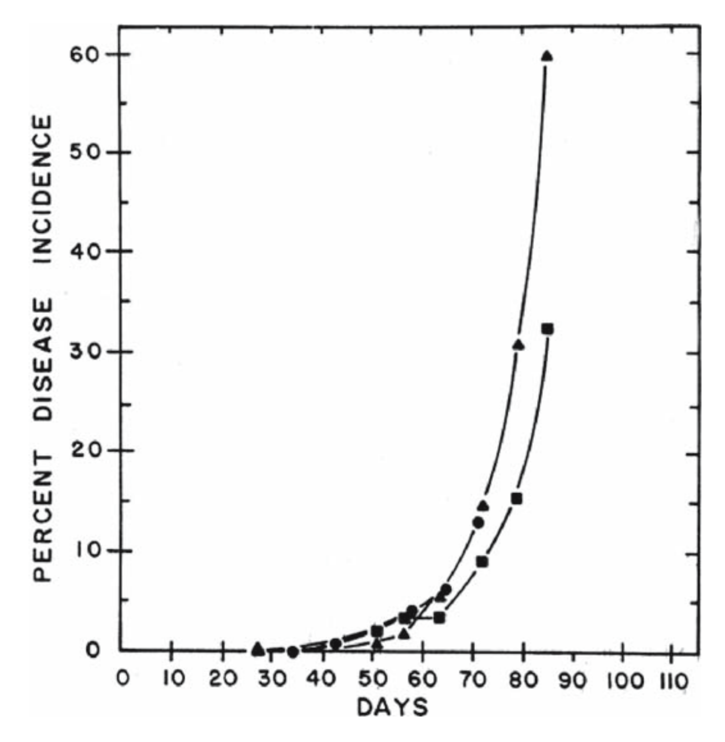


Fig. 16.2.1.3 Representative disease progress curve for lettuce drop at three initial inoculum levels of *Sclerotinia minor* at planting. (▲) A field with mean of 10.48 sclerotia per 100 cm^2 of soil; (■) A field with a mean of 6.36 sclerotia per 100 cm^2 of soil; (●) A field with a mean of 1.84 sclerotia per 100 cm^2 of soil (Adapted from the publication of Dillard and Grogan, 1985. With permission)

between fields (Jarvis and Hawthorne, 1972). Mycelial growth from plant to plant may occur and sclerotia may spread within and between fields in irrigation water, machinery, etc. (Abawi and Grogan, 1979). Sclerotia of *S. minor* in contact with the main lettuce stem on the soil surface causes highest percentage of infection, if located >1 cm from the plant, no infection takes place. Sclerotia in contact with the main root at greater depth are less effective in causing infection (Imolehin and Grogan, 1980).

16.2.3 Factors Affecting Host Infection and Disease Development

Infection of lettuce by *S. minor* results from mycelial germination of sclerotia (Adams and Tate, 1976; Hawthorne, 1974; Marcum et al., 1977), thus, inoculum density of germinable sclerotia in soil and the prevalence of conditions that favour

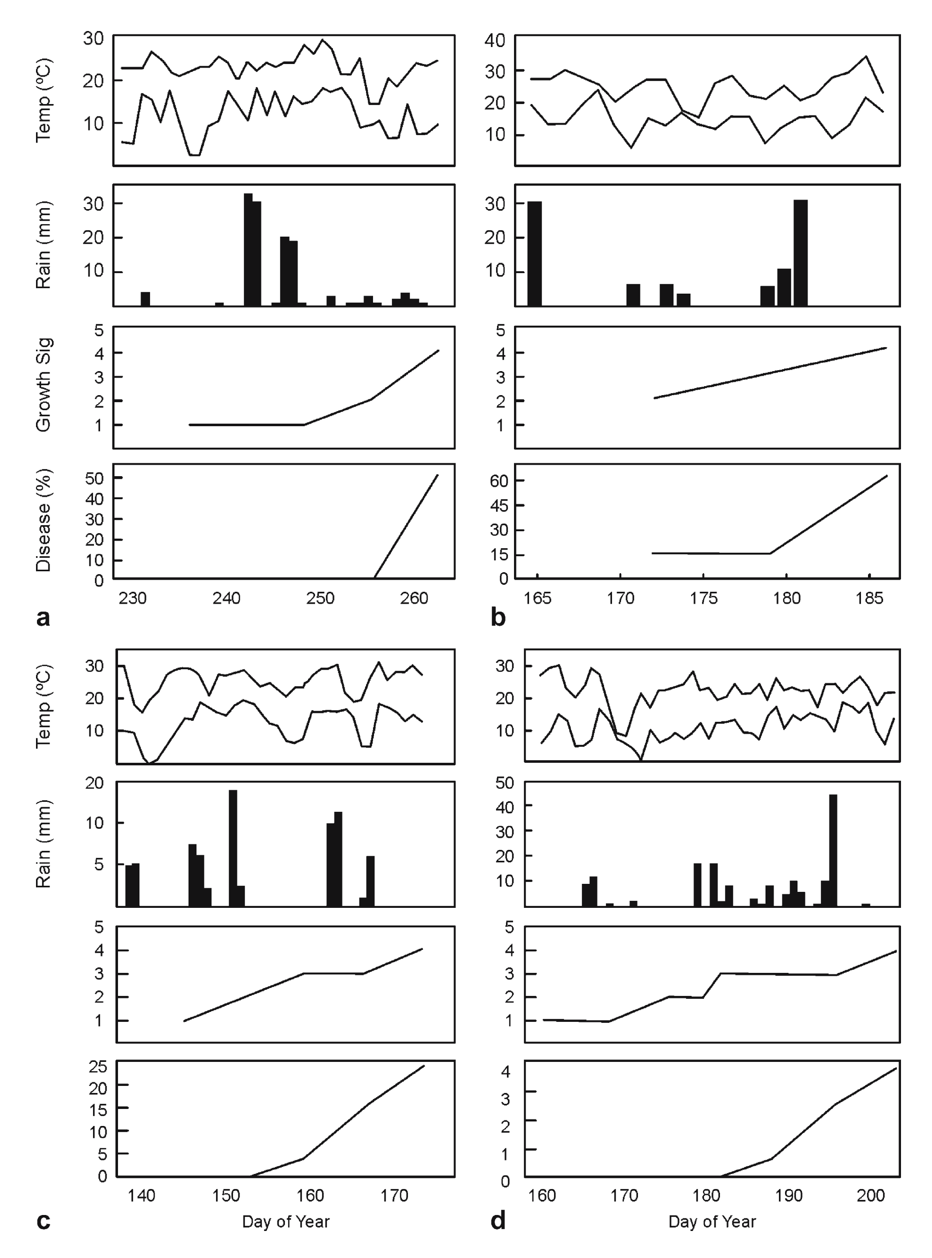


Fig. 16.2.1.4 Incidence of lettuce drop disease (Disease %), crop growth stage (Grwth stg.), rainfall (Rain mm) and maximum and minimum daily temperature (Temp. °C) in crops 1(**a**), 4 (**b**), 5 (**c**) and 7(**d**) (Adapted from the publication of Melzer and Boland, 1994. With permission)

Table 16.2.1.1 Indices of dispersion and best fit probability distribution for the sclerotial populations of *Sclerotinia minor* in 15 naturally infested field plots (Adapted from the publication of Dillard and Grogan, 1985. With permission)

Plot[a]	S^2x[b]	K[c]	Model with best fit[d]	Probability of exceeding x^2 value
A	1.7	12.4	NB	0.19
B	1.7	17.3	NB	0.62
C	1.9	8.2	NB	0.44
D	1.9	2.2	LWZ	0.75
E	1.4[e]	5.0	NB	0.95
F	1.9	7.4	NA	0.47
G	1.5	15.6	NB	0.66
H	1.2[e]	25.7	PB	0.71
I	1.2[e]	27.9	PB	0.82
J	1.6	9.9	–	–
K	1.3[e]	21.6	NB	0.59
L	2.7	7.5	NB	0.78
M	1.9	8.8	NB	0.24
N	2.9	5.9	NB	0.50
O	2.4	7.1	NB	0.90

[a] Values for plots A to E are from 50 soil samples per plot. Values for plots F to O are from 100 soil samples per plot

[b] Variance-to-mean ratio

[c] Dispersion parameter of the negative binomial distribution

[d] Discrete frequency distribution models: NB = negative binomial, LWZ = logarithm c with zeros, NA = Neyman type A, PB = Poisson binomial, – no significant fit to the discrete frequency distributions tested

[e] Not significantly ≥1, hypothesis of randomness not disproved

Table 16.2.1.2 Results of ordinary runs analysis to determine the pattern of lettuce plants infected by *Sclerotinia minor* (Adapted from the publication of Dillard and Grogan, 1985. With permission)

Plot	Observed runs	Expected runs	Standard deviation	Z[a]	Pattern[b]
A	91	93	6.7	−0.2	Random
B	97	91	6.6	1.0	Random
C	75	83	6.0	−1.3	Random
D	49	46	3.6	0.9	Random
E	47	46	3.3	0.3	Random

[a] Standardized variable; large negative values indicate clustering

[b] Analysis combines adjacent rows. Analysis of individual rows demonstrated random patterns, except for Row 2 in Plot C where a significant aggregation of diseased plants was detected

sclerotial germination influence the incidence of lettuce drop. Lettuce drop is most severe when cool and moist weather conditions prevail (Brown and Butler, 1936; Moore, 1955) or irrigation is excessive during the growing season and especially near harvest time. In addition, lettuce drop is more prevalent in low and poorly drained areas of the field. The disease incidence is significantly higher under furrow irrigation than under subsurface drip irrigation because of aggregation of

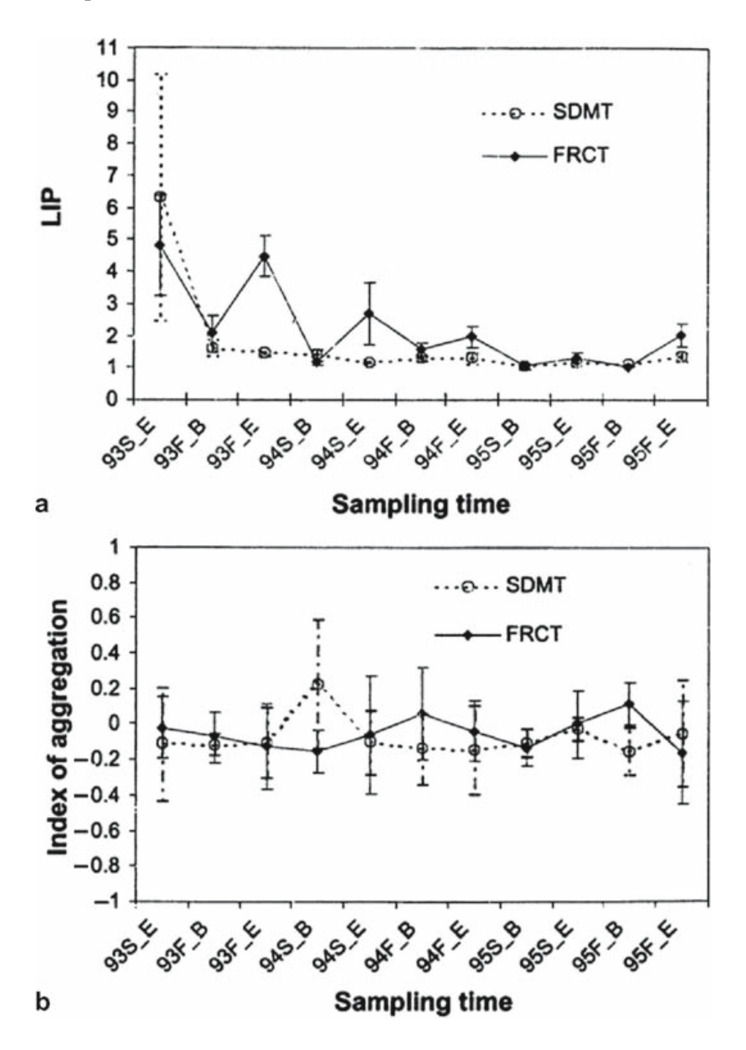


Fig. 16.2.1.5 Aggregation of *Sclerotinia minor* sclerotia under subsurface drip with minimum tillage (SDMT) and furrow irrigation with conventional tillage (FRCT) (Adapted from the publication of Wu and Subbarao, 2003. With permission)

sclerotia (Wu and Subbarao, 2003). However, the incidence of lettuce drop is greater when soil moisture is allowed to fluctuate from 100 to 30 per cent field capacity as compared to 100 to 80–90 per cent field capacity (Adams and Tate, 1975). It is suggested that drying of sclerotia at or near the soil surface stimulates germination and infection when soil moisture is adjusted again to near field capacity (Beach, 1921; Smith, 1900). Although sclerotial germination is best at soil moisture near field capacity (−300 mb), considerable germination occurs at a soil water potential of −2 bars at soil water potentials of 0, −0.05, −0.2, −1.0, −2.0 and −5.0

bars, sclerotial germination is 5, 95, 65, 36, 9 and 2 per cent respectively (Abawi and Grogan, 1979). Nevertheless, free moisture still may be required for successful host infection as is the case with *S. sclerotiorum* on beans. It is demonstrated under field conditions that significantly fewer drops occur on a lettuce cultivar with an upright growth habit than on the cvs. Butter Crunch and Great Lakes. The lower leaves of the latter two cvs. are close to or in contact with the soil. All three cvs. are equally susceptible to infection by *S. minor* under greenhouse conditions when artificially inoculated. Thus, it is concluded that the lower leaves of both Butter Crunch and Great Lakes, in addition to being in contact with the soil, modify the soil microclimate by making it wetter and cooler and thus, more inducive to sclerotial germination. In fact, germination of sclerotia is observed only a few centimeters from the base of the plant and only under the lower leaves (Hawthorne, 1974). Thus, the escape of cvs. of lettuce under field conditions probably is due to reduced soil moisture and possibly to higher temperatures in the microclimate. *Sclerotinia* spp. generally are favoured by low temperatures with a favourable infection temperature of about 10–25°C (Abawi and Grogan, 1979).

Although infection of lettuce by *S. minor* may occur at any time during the growing season, most infection becomes evident by death of plants (drop) after head formation and as the crop approaches maturity (Adams and Tate, 1975; Hawthorne, 1974; Marcum et al., 1977). Mycelium produced by germinating sclerotia infects lower leaves and crown tissues and death usually follows within one week. Similarly, below ground infections of the main root result in plant death within 7–14 days. However, infection originating on secondary roots progresses more slowly and a three week or longer incubation period may be required for symptom expression and plant death (Abawi and Grogan, 1979). It is postulated that primary infections are initiated by ascospores, whereas secondary infections are associated with inocula (Jarvis and Hawthorne, 1972). Later on Hawthorne (1974, 1975), however, suggested that ascospores are not likely to be the main source of inoculum in New Zealand. Under moist conditions, infected leaf and crown tissues become covered with cottony mycelium of *S. minor*, especially near the soil line. Numerous sclerotia then are produced on the mycelial mat. Sclerotia are the main surviving structures as the mycelium probably is short lived in soil. The newly produced sclerotia, after undergoing a dormancy period must also undergo drying before it will germinate. Functionally mature sclerotia in soil may germinate and produce infection or may produce secondary sclerotia (Adams, 1975; Beach, 1921).

Factors that are known to influence the severity of lettuce drop caused by *S. minor* include the growth stage of lettuce, air temperature and soil moisture. The growth stage of lettuce is an important factor because disease is normally not observed until the crop is heading (Beach, 1921; Hawthorne, 1974). Cool air temperature and moist soil appear to favour disease development (Abawi and Grogan, 1979). During 1989–1992, seven lettuce crops were monitored weekly by Melzer and Boland (1994) for crop growth stage and incidence of lettuce drop caused by *S. minor*. Daily maximum and minimum air temperature and daily rainfall were measured at a nearby regional weather station. Lettuce drop normally develops

after plants have begun to head (crop growth stage 3) and rainfall 7–46 mm result in moist soil conditions that coincide with four to ten days of daily minimum and maximum air temperatures of 5–18°C and 14–25°C respectively. Canopy microclimate analysis in 1992 determined that the influence of a crop canopy on microclimate within the crop is minimal (Fig. 16.2.1.4). The linear and monomolecular models are most appropriate for modeling three disease progress curves that contains three none zero data points. Two types of epidemics are observed; those which are rapid, with maximal disease being reached in 7 days, and those which are slower with disease increasing for 14–28 days before harvest depending on the amount of sclerotia present in the field (Melzer and Boland, 1994). However, Hao and Subbarao (2005) classified *Sclerotinia* infections on the basis of symptoms. One which is caused directly by eruptive germination of sclerotia (Type I) and second by airborne ascospores (Type II). Regardless of the analytical method employed, disease incidence with type I infection (Fig. 16.2.3.1) shows an aggregated pattern in a majority of the fields and random pattern in the fields where incidence is low. Lettuce drop incidence in fields with type II infection (Fig. 16.2.3.2) is erratic in time and peaks within a very short time. The source of inoculum and the type of infections they cause are most likely to determine spatial patterns of lettuce drop in the field.

16.3 Peanut Rot

In peanut, infection is myceliogenic, i.e., the mycelium grown from the sclerotia cause infection on the pegs or lateral branches near the soil. Low temperature (10–25°C) and high soil moisture favour infection and the disease become severe when there are more cold days in a growing season. The possibility of ascospores being the source of primary and secondary infection in Oklahoma (USA) has been observed (Wadsworth, 1979). Rapid colonization of the plant is facilitated by defoliated leaves fallen on the ground or senescent leaves while on the plants and touching the soil surface or remoistened dried peanut leaves (Porter, 1980a; Hau et al., 1982). Plants injured during intercultural operation are predisposed to infection (Porter and Powell, 1978). Peanut plants sprayed with captafol or chlorothalonil (0.56–2.24 kg/h) are affected more severely by the disease (Beute et al., 1975; Porter, 1977, 1980b; Porter and Lankow, 1981) possibly because plants sprayed with these chemicals favour more production of oxalic acid.

Myceliogenic sclerotial germination of *S. minor* and growth as well as infection and colonization of peanut tissue are optimum at 20–25°C. Ninety five to 100 per cent RH for more than 12 h is necessary for germination of sclerotia. At 100 per cent RH for varying periods of time 80 per cent sclerotia germinate. Lateral branch or main branch tissues are colonized by *S. minor*. Similarly, the infection rate of young, juvenile tissues is significantly greater than that of maturing plant tissues (Dow et al., 1988b). Plant canopies affect soil temperature, soil moisture, amount and duration of leaf wetness, canopy RH and canopy temperature. The distribution of leaf area near the soil surface, the plant canopy structure and the plant

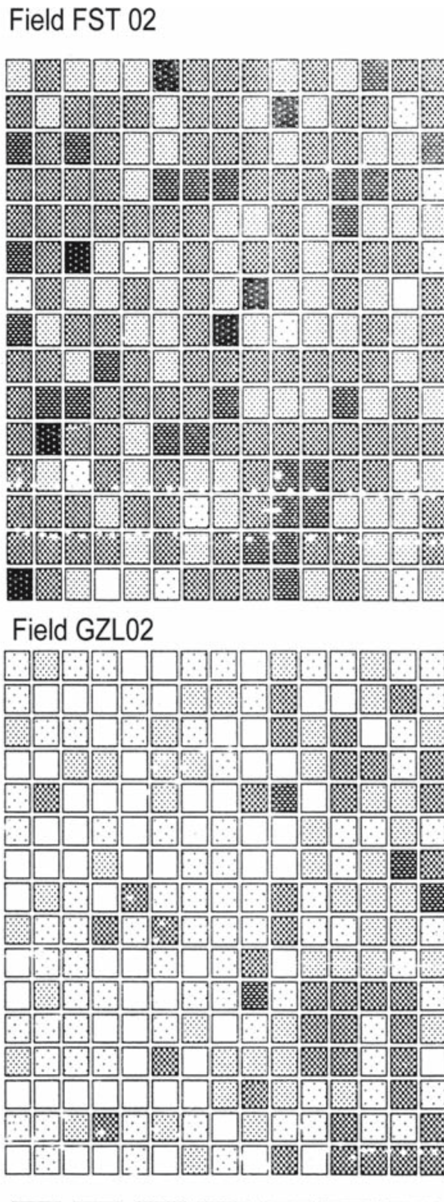


Fig. 16.2.3.1 Distribution of lettuce drop incidence (%) caused by *Sclerotinia minor* in two commercial lettuce fields, representing type 1 infection, in California. Each small square represents incidence in a 2-by-2 m quadrate, with about 24 plants each. The different pattern represents incidence classes shown in the legend (Adapted from the publication of Hao and Subbarao, 2005. With permission)

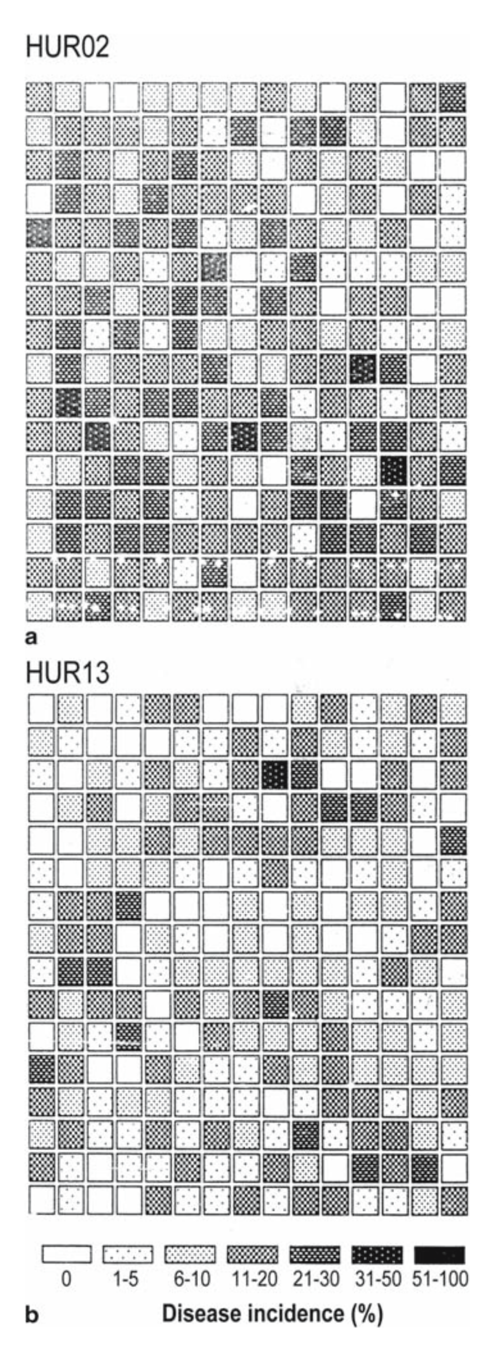


Fig. 16.2.3.2 Distribution of lettuce drop incidence (%) caused by *S. sclerotiorum* in two commercial lettuce fields, representing type II infection, in California. (**A**) Data from field HUR02 and (**B**) Data from field HUR 13. Each small square represents incidence in a 2-by-2 m quadrate, with about 24 plants each. The different pattern represents incidence classes shown in the legend (Adapted from the publication of Hao and Subbarao, 2005. With permission)

canopy density associated with the growth habit of the peanut plant are considered factors in determining microclimate effects on *Sclerotinia* blight development. Following infection by *S. minor*, the number of infection foci and disease development is reduced in the thinned canopy (Dow et al., 1988a, b). Rainfall is determinant in triggering the onset of outbreaks of *S. sclerotiorum* blight of peanut in Argentina. The new sclerotia germinate carpogenically in the some season in which they are formed. It is due to abundant and frequent rains conditioning the sclerotia resulting into two biological cycles of the pathogen in the one growing season (Marinelli et al., 2004). *Sclerotinia* blight of peanut caused by *S. minor* generally becomes severe only after vines meet in the row middle and a dense canopy develops. Dense foliage appears to support a microclimate conducive to the colonization of peanut limbs by *S. minor* (Butzler et al., 1998).

16.4 Sunflower Rot and Wilt

The susceptibility of sunflower plants is low at first, reaching maximum at budding followed by a decline as the flowers develop and again becomes maximum at fertilization. Incidence of the disease decreases as the head matures. However, significant plant wilting at the late bud stage may also be observed (Auger and Nome, 1971). A high incidence of the disease, in some cases, may be traced to the history of the field. In the severe epidemic of the disease on sunflower in 1971 in southern Manitoba, the crop histories of 19 fields with varying amounts of disease showed that the most severe disease occurred when the crops had been grown in two or three seasons in the five year period from 1966 to 1970 (Zimmer and Hoes, 1978; Hoes, 1971).

Sunflower wilt incidence is highest when sclerotia are buried next to seed and decreases with increasing distance between (Tables 16.4.1–16.4.3) sclerotia and seed (Huang and Hoes, 1980). Dense plant spacing increases the incidence of the disease in sunflower (Bisby, 1921; Jones, 1923; Young and Morris, 1927). Closer plant spacing favours faster spread of wilt (Hoes and Huang, 1976; Huang and Hoes, 1980). If the sclerotia and seeds are deposited close together, early infection develops and the spread of the disease is favoured by dense planting when sunflower is cropped within row spacing of 10 cm between plants. The efficiency of plant to plant spread of the disease (Fig. 16.4.1) by *S. sclerotiorum* is reduced by decreasing the sunflower population (Huang and Hoes, 1980).

Generally high fertility and moisture supplies favouring vegetative growth of the plant also favour root infection and development of wilt. Abundant rainfall favours disease (Krexner, 1969). Long periods of precipitation with short rainless intervals during which the heads dry, increase the incidence of head rot. In the Tambou region of the Soviet Union, the optimum conditions for the ascus state occur in July-August in wet weather at 18–22°C. Mass spore discharge occurs at 20–22°C and lasts for 18–20 days (Rogozheva and Kochenkova, 1982). A short photoperiod predisposes the hypocotyls to infection (Orellana, 1975).

Table 16.4.1 Effect of plant spacing on time and efficiency of *Sclerotinia sclerotiorum* to spread from primary infection locus[a] (PIL) and cause wilt in sunflower (Adapted from the publication of Huang and Hoes, 1980. With permission)

	Within row plant spacing (cm)			
Variables	10	20	30	40
Efficiency of spread[b]	9/12	3/7	5/9	1/9
Time (wk) for plant-to-plant spread of pathogen				
Range	1–5	1–5	3–7	–
Mean	1.5	2.6	4.3	4.0
No. of wilted plants neighboring PILs				
Range[c]	0–8	0–5	0–2	0–1
Mean[d]	3.3	1.7	0.7	0.1

[a] Primary infection locus = First usually a series of plants to develop wilt as a result of infection from sclerotia
[b] No. PIL/total, from which pathogen spread to adjacent plants
[c] Based on all PIL of each plant spacing
[d] Based on totals of 40, 12, 6 and 1 plant and all PIL of respective spacings

Table 16.4.2 Effect of vertical distance between seed and sclerotia of *Sclerotinia sclerotiorum* on incidence of wilt in sunflower[a] (Adapted from the publication of Huang and Hoes, 1980. With permission)

Distance of sclerotia above or below seed[b] (cm)	Wilted plants[c] (%)
4 (above)	18[bc]
0 (seed level)	52[a]
5 (below)	16[bc]
15 (below)	21[b]
25 (below)	8[c]

[a] Seed, 5 cm deep; rows, 90 cm apart within row spacing, 30 cm
[b] Ten sclerotia per site of infestation
[c] Data based on 126 plants per treatment; six replicates (Duncan's multiple range test)

Table 16.4.3 Effect of horizontal distance between seed and sclerotia of *Sclerotinia sclerotiorum* on incidence of wilt in sunflower[a] (Adapted from the publication of Huang and Hoes, 1980. With permission)

Distance of sclerotia above or below seed[b] (cm)	Wilted plants[c] (%)
0	57[a]
10	18[b]
20	0[c]
30	3[b]

[a] Rows, 90 cm apart within row spacing, 30 cm
[b] Five sclerotia per site deposited at seed level (5 cm). At 10 cm one site; at 10, 20 and 30 cm four site in crosswise pattern
[c] Data based on 50–60 plants per treatment; six replicates (Duncan's multiple range test)

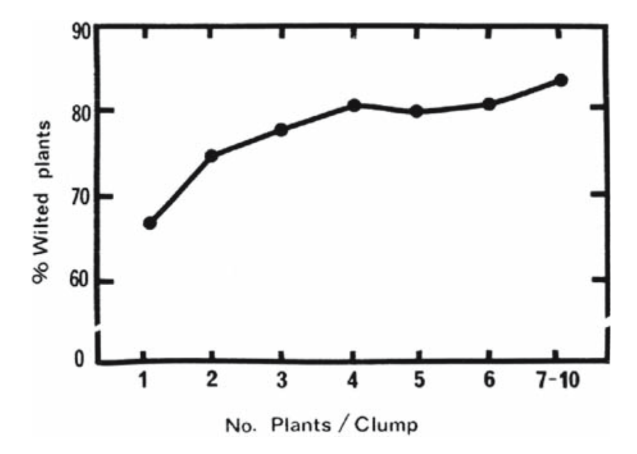


Fig. 16.4.1 Effect of plant density on incidence of sunflower wilt caused by *Sclerotinia sclerotiorum*. Data based on 912 plants occurring singly; the number of plants belonging to clumps varied from 132 in clumps of six plants to 780 in clumps of two (Adapted from the publication of Huang and Hoes, 1980. With permission)

In France, Payen (1983), Lamarque (1983) and Perez et al. (1989) established that *Sclerotinia* epidemics of sunflower have significant correlations with high humidity (100 per cent) and surface wetness duration for 24 h. According to Sedun and Brown (1987), leaf wetness is essential for infection with maximum infection after a 72 h dew period exposure.

According to Nelson et al. (1989), the differences in rates of disease progress between plant populations has no apparent effect on seed yield. Rates of disease progress are positively correlated with inoculum density, but not with precipitation and temperature. Sclerotia of *S. sclerotiorum* survive up to five years in soil where a sunflower crop has been grown. Sclerotial population declines gradually from spring through summer to autumn (Milinko et al., 1989). Predictive modeling using CLIMEX software suggested conditions suitable for carpogenic germination of *S. minor* in southern Australia (Ekins et al., 2002).

16.5 Soybean Stem Rot

The epidemiology of *Sclerotinia* stem rot of soybean appears to be similar to that of white mould of beans (*Phaseolus vulgaris* L.). *S. sclerotiorum* survives as sclerotia that must be at or within 5 cm of the soil surface to produce apothecia. Apothecia optimally form at soil temperatures of 15–18°C and a water matrix potential of –0.25 bars for 10–14 days. Apothecia produce ascospores that are forcibly ejected and disseminated by air currents to the host surface. Ascospores released from apothecia are the primary source of inoculum for disease in soybean (Grau et al.,

1982; Cline and Jacobsen, 1983). In the absence of an exogenous nutrient source, ascospores on intact host tissues produce a short and usually sub-polar germ-tube but only young host tissues are penetrated by the infection hypha arising from the germinated spore. There is a hypersensitive response by cells to penetration and generally the fungus remains restricted to these cells, though it continues to grow within them. Water soaked lesions characteristic of successful infections only develop when many individual infection sites coalesce following inoculation with high concentrations of ascospores. Flowers or parts of flowers provide a suitable nutrient base for initial colonization from ascosporic inoculum. Mycelium extending from this base initiates infection of intact host surfaces (Sutton and Deverall, 1983). Disease symptoms are associated with post-flowering crop stages, the initial colonization of senescent tissues before plant infection and the appearance of lesions in leaf axils. Seasonal rainfall, irrigation management, air temperature and extended periods of leaf wetness affect disease incidence and severity (Cline and Jacobsen, 1983; Grau et al., 1982; Grau and Radke, 1984; Phipps and Porter, 1982). In Crotia, the occurrence of white mold in velvetleaf, ragweed and gough cocklebur in soybean fields increases inoculum density of *S. sclerotiorum* in soil (Vrandecic et al., 2003).

Quantitative information on the relationship between apothecia or ascospores and disease development is limited. The frequency of apothecia in various crops ranges from 0–40 apothecia per square meter (Henderson, 1962a; Gabrielson et al., 1973; Letham et al., 1976; Schwartz and Steadman, 1978; Williams and Stelfox, 1980a) and ascospore dispersal ranges from 35 m to several kilometers (Brown and Butler, 1936; Suzui and Kobayashi, 1972a, b). There is little information on the relationship of numbers or location of apothecia to the development and severity of stem rot of soybean along with the spatial pattern of apothecia or disease in cultivated fields. Apothecia and disease are both spatially aggregated and are most consistently described by the negative binomial distribution, although the Poisson-binomial and logarithmic with zeros distributions sometimes gives significant fits to data. Numbers of apothecia and disease incidence are correlated within quadrates of 1.4, 36 and 108 m^2. The disease incidence is determined primarily by inoculum produced within the field (Boland and Hall, 1988b, c).

The internally infested soybean seeds are potential means for field to field dissemination of the pathogen. There is a higher percentage of sclerotium production in the soil from the internally infected soybean seeds (Yang et al., 1998). Canopy temperatures less than 30°C and plant wetness for 12–16 h recurring on a daily basis or continuous surface wetness for 42–72 h are environmental conditions needed for disease development. In Wisconsin, mean minimum temperature of 20°C/34°C two weeks before and two weeks after flowering resulted in the complete absence of *Sclerotinia* stem rot. The disease was prevalent the previous and following year at the same location when mean minimax temperatures were 12°C/22°C and 18°C/30°C respectively (Grau and Radke, 1984). The crop canopy can greatly affect environmental conditions needed for optimum activity by the pathogen and for subsequent disease development. Thus, cultural practices that modify the canopy environment have a potential impact on the incidence and severity

of *Sclerotinia* stem rot. A 65 per cent increase in incidence of *Sclerotinia* stem rot and a 42 per cent reduction in yield have been measured for soybean cvs. grown in narrow (18–38 cm), as compared to wide row widths (75–90 cm). Plant population as well as row width also affects the incidence of disease. Sprinkler irrigation is another cultural practice that can greatly modify the canopy environment and lead to greater disease, especially if applied when flowering is occurring at the lower nodes (Grau and Radke, 1984).

16.6 Rapeseed and Mustard

Species of *Sclerotinia* can function either as soil borne or air borne pathogen. Infection of above ground plant parts result from ascosporic inoculum whereas soil borne infection may result either from ascospores or sclerotia. Below ground infection however, results from mycelial germination of soil borne sclerotia. Continuous moisture for about ten days is required for apothecial development and even a slight moisture tension prevents apothecial formation. Temperature of 6–10°C during March and April and high soil moisture until the apothecia have developed, with subsequent changeable weather favours infection. Ascospore release and petal fall should occur at the same time (Kruger, 1980). Morrall and Dueck (1982, 1983) have reported severe infections in the fields with few or no apothecia. Clarkson et al. (2003) reported that apothecia of *Sclerotinia sclerotiorum* are produced at an optimum temperature of 15°C and ascospores survive a wide range of conditions but high temperature and humidity reduces the viability. No apothecial initials are produced at either 30°C or 5°C. Approximately 48–72 h of continuous leaf wetness is required for infection by ascospores. The infection by *S. sclerotiorum* in yellow sarson and in *B. campestris* var. toria aggravates by low temperature, heavy rain fall and closer spacing (Saxena and Rai, 1988).

The pattern of petal fall and petal deposit on leaves suggests that the crop is most vulnerable to infection towards the end of flowering about 25 days after the beginning of flowering in the UK (Mc Cartney et al., 2001b). The role of extrinsically produced ascospores in causing disease in rapeseed fields may therefore be of considerable importance (Morrall and Dueck, 1982, 1983; Williams and Stelfox, 1979, 1980b). Accordingly, ascospore concentrations above the crop canopy and on plant surfaces might reflect the disease potential in a crop better than the density of apothecia in the field. Geigel and Morrall (1986) demonstrated significant relationship between petal infestation at early bloom and disease incidence. Infested petals and disease are regularly found when apothecia are absent, thereby demonstrating the rate of extrinsically produced ascospores in the infestation of crops. Flowers of rapeseed from the time they expand retain their petals on an average for six days. During this period, the petals "in situ" are contaminated by ascospores of *Sclerotinia*. Infection takes place preferentially on senescent petals because young petals are actually resistant to a certain extent being colonized truly because the spores are not exposed to conditions that are favourable to their germination on the young petals. The senescent petals are the most easily colonized (Mc Lean, 1958b) and do provide

Table 16.6.1 *Sclerotinia* rot incidence (mean of infected plants/pot) of mustard crop in various sequential cropping systems (Adapted from the publication of Shukla, 2005. With permission)

		Nitrogen levels (kg/ha)					
S. No.	Cropping system	0	40	80	120	160	Mean
1.	Blackgram-mustard	1.25	1.38	2.25	4.13	5.5	2.90*
2.	Sesame-mustard	1.75	2.0	2.13	3.13	13.63	4.52*
3.	Bajra-mustard	0.38	0.25	0.75	1.63	5.15	1.65
4.	Dhaincha-mustard	1.75	1.63	4.88	7.13	8.75	4.82*
5.	Sunflower-mustard	1.25	1.50	3.38	6.75	22.63	7.10*
6.	Fallow-mustard	1.25	1.25	3.50	7.13	14.13	5.45*
7.	Mean	1.35	1.34	2.84*	4.98*	11.63*	4.41

*C.D. at 5% Significance
Cropping system (CS) 1.01 CS × N levels 2.96
Nitrogen levels (N levels) 1.21 N levels × CS 2.84

the ascospores with a source of carbon which permits their germination (Purdy, 1958). The hyphae which develop subsequently play a very important role in the initiation of infection (Kapoor, 1983). Indeed, dead petals often stick to leaves and this allows the disease to become established (Brun et al., 1983).

Nitrogen fertilization is known to increase the incidence of the disease (Brun et al., 1981). However, higher nitrogen in the plant is reported to decrease the susceptibility (Iwata and Igita, 1972). Increasing the level of N (60–90 kg/ha) in soil leads to increase *Sclerotinia* rot of mustard from 26.3 to 37.7 per cent. Sulphur decreases the disease incidence from 33–24 per cent when applied at 40 kg/ha (Gupta et al., 2004a). Mustard sown after sunflower, Dhaincha and sesamum contracts (Table 16.6.1) more disease (Shukla, 2005b).

Late seeding may reduce the disease by shortening the overlap between phenolic susceptibility of the plants and exposure to maximum ascospore load (Morrall and Dueck, 1982). The herbicide Barban, when sprayed on the rapeseed crops, increases its susceptibility to infection by *S. sclerotiorum*, possibly through altering the physiology of the plant as the herbicide has no inhibitory effect on the pathogen (Berkenkamp and Friesen, 1973). *B. juncea* plants, when artificially inoculated with the pathogen and sprayed with different sulfur compounds, show varying degrees of development of the disease with the susceptibility of the plants being influenced more by ammonium-sulfate. Thiourea spray, however, shows less intensity of the disease (Dhawan et al., 1979).

While studying clonal dispersal and spatial mixing of *S. sclerotiorum* isolates from rape fields in Canada, it has been observed that there is spatial mixing of ascospore inoculum from resident or immigrant sources (Kohli et al., 1995).

16.7 Forage Legume Rot

Sclerotinia crown and stem rot (SCSR) caused by *S. trifoliorum* Erikss. is a widespread and destructive disease of forage legumes in north temperate regions of the World. Crown and stem rot of alfalfa caused by *S. sclerotiorum* was reported by

Gilbert (1987) in southeastern Washington in 1982. Primary infection occurs on leaves from apothecia that develop from over summered sclerotia (Djikstra, 1964; Loveless, 1951; Valleau et al., 1933; Williams and Western, 1965b). Secondary infection of other plant parts and adjacent plants occurs in winter and spring by mycelia that spread from leaf lesions following freezing damage (Djikstra, 1966), prolonged hydration (Loveless, 1951), or death of leaves (Valleau et al., 1933). New sclerotia form within and beneath patches of plants that are killed by the disease. Incidence and severity of SCSR varies greatly from year to year (Lester and Large, 1958) and is related to winter temperature and rainfall (Lester and Large, 1958; Loveless, 1951; Valleau et al., 1933). In North Carolina, apothecia form in fall and early winter and mycelial spread in crimson clover occurs from October to March (Wolf and Cromwell, 1919). In Kentucky, apothecia form mainly in October and mycelial spread in red clover occurs from January to March (Gilbert and Bennett, 1917; Valleau et al., 1933). According to Pratt and Knight (1982), apothecia develop most frequently during December from sclerotia collected in the field, stored air-dry for six to seven months at 25°C and 36°C and buried at <1 cm in September and October. Fewer apothecia develop from sclerotia stored at 4°C, buried at >3 cm, or added to soil after October. Disease patches appear from January to March and reach maximum size by April. For both seedling and older plants Ladino clover, infection at 16°C and 21°C is significantly greater than at 27°C. Better infection is obtained at 90 per cent than at 50 per cent RH (Kreitlow and Sprague, 1951). Pollen grains of alfalfa are infected by *S. sclerotiorum* by direct hyphal penetration through the equatorial germinative pores or through the exine and intine layers of the pollen wall without formation of infection cushions or appressoria. After penetration, hyphae ramify within the pollen grains, causing plasmolysis of the cytoplasmic membrane and eventual disintegration of the pollen cytoplasm (Huang et al., 1997b).

While studying kinetics of the ascospore production of *S. trifoliorum*, Raynal (1990) recorded maximum discharge of spores at 10–15°C under high RH. Ascospore discharge is maximum between 1,500 and 1,600 h and extremely low at night and ceases at 1,900 h. According to Raynal et al. (1991), the pathogen sporulate only in October and November and develops rot on the leaves and crown in winter and early spring under conditions of continuous moisture and temperature of 10–15°C. The disease can be highly destructive on young stands sown in the autumn.

Three species of slugs, *Deroceras reticulatum*, *Arion fasciatus* and *A. subfuscus* which have fed on sclerotia or apothecia of *S. trifoliorum* transmit the disease of white clover plants (Shakeel and Mowat, 1992).

16.8 Pea White Rot

Humid weather and temperature range of 15–25°C seems to be the prime requisite for infection (Gray and Findlater, 1960). Moore et al. (1949) and Partyka and Mai (1962) reported that high humidity favours disease development. According to

Singh (1991a), the disease incidence and lesion size are positively correlated with temperature, relative humidity, per cent rainy days and total rainfall. For disease incidence and lesion growth per cent rainy days seems to be more important as compared to total rainfall. Soil temperature has negative correlation with disease incidence. Maximum disease incidence and lesion size has been recorded during the month of March at 14°C. Disease declines significantly above 20°C. A temperature range of 10–20°C is most congenial for disease development.

16.9 Carrot Rot

The epidemiology of *Sclerotinia* rot of carrot is distinct from several other *Sclerotinia* diseases because it is characterized by two interconnected epidemics involving pre-harvest foliar injection in the field and post-harvest root injection in storage (Kora et al., 2003). Apothecia are first detected in the crop in early August to mid-September, after the carrot canopy closes and after 7–11 days with soil matric potentials between –0.1 and –0.4 bars and soil temperatures between 14°C and 23°C. Ascospores are first detected in mid-July to mid-August, usually before apothecia are observed in the crop and after 7–12 days with soil matric potentials between –0.1 and –0.3 bars and air temperatures between 15°C and 21°C. The numbers of apothecia and ascospores are positively correlated with soil matric potential. Pre-harvest epidemics starts in mid-August to mid-September, after the closure and lodging of the canopy, after the senescing of the leaves in the crop as well as appearance of ascospores on the soil, and rain initiated leaf surface wetness for 12–24 h per day. Disease incidence is negatively correlated with air and soil temperatures. Post-harvest epidemics in storage follows pre-harvest epidemics in the field, but not all pre-harvest epidemics results in disease in storage. It is suggested that severe epidemics of *Sclerotinia* rot of carrot can occur in storage when disease in the field progresses rapidly and is associated with soil matric potentials of >= –0.2 bars and leaf wetness of >= 14 h per day, particularly close to harvest (Kora et al., 2005b).

16.9.1 The Pre-harvest Epidemic

Early workers postulated that primary infection of carrot is initiated by mycelium arising from myceliogenically germinating sclerotia in soil (Lauritzen, 1932; Mukula, 1957), or by air borne ascospores of *S. sclerotiorum* (Rader, 1952). However, there is insufficient evidence on the epidemiological significance of these forms of inoculum in the field.

Study in Manitoba, Canada demonstrated that infection of carrots is initiated primarily through mycelial colonization of leaf and when tissues close to or indirect contact with germinating sclerotia located on or near the soil surface (Finlayson et al., 1989). In controlled experiments, foliar infection occurs more rapidly and

disease in storage is greater when mycelial inoculum is placed near carrot foliage that is in contact with soil then when mycelium is placed near roots. Mycelium of *S. sclerotiorum* is capable of infecting carrot leaves even after advancing a distance of 0.5–1 cm through soil less growing medium. In contrast, foliar applied ascospores require at least 11 days of continuous leaf wetness to produce disease in foliage and stored roots. In field conditions, inoculation with ascospores suspension does not cause disease on foliage or stored roots, as leaf wetness duration is never sufficient for infection to occur (Finlayson et al., 1989). However, there is no indication in these studies about the role of differential susceptibility of leaf tissue to ascospores with respect to their development stage or position within the canopy.

Studies in the UK indicated that airborne ascospores produced in autumn are the most important inoculum in initiating epidemics of *Sclerotinia* rot of carrot in the field (Geary, 1978). The presence of apothecia within carrot crops has been consistently associated with the initial appearance of symptoms of *Sclerotinia* rot of carrot (Couper, 2001; Geary, 1978). However, the absence of apothecia in some infected carrot crops (Geary, 1978) suggests the presence and relative importance of extrinsic sources of ascospores, as reported for epidemic of white mould in beans (Abawi and Grogan, 1975; Boland and Hall, 1987), lettuce drop (Patterson and Grogan, 1985) and *Sclerotinia* stem rot in rapeseed (Morrall and Dueck, 1982). In optimum controlled conditions, treatment with airborne ascospores initiates foliar infection only when carrot plants are at the seven to eight leaf stages or later and have atleast one senescing leaf at the time of inoculation. Symptoms develop only on senescing leaves and appear three to four days after inoculation. Infection spread to the crown and new sclerotia formed on the foliage about 14 days after inoculation (Geary, 1978).

These studies suggest that airborne ascospores and soil borne hyphae are important primary inocula for the epidemiology of *Sclerotinia* rot of carrot (Fig. 15.1) and their prevalence may depend on the region, environmental conditions and cropping system. Although *S. sclerotiorum* is capable of infecting by hyphae or ascospores, it appears to function primarily by producing apothecia and mycelial germination from sclerotia contributes minimally, if at all to the development of epidemics (Abawi and Grogan, 1979). In other crops, *S. sclerotiorum* infects senescing plant tissues by means of ascospores (Abawi and Grogan, 1975; Cline and Jacobsen, 1983; Huang and Hoes, 1980; Morrall and Dueck, 1982; Patterson and Grogan, 1985) and direct infection by sclerotia is not considered important except for the basel infection of sunflower (*Helianthus annuus*) (Huang and Dueck, 1980) and tomato (*Lycopersicon esculentum*) (Letham et al., 1976) and infection of bean leaves in contact with the soil surface (Tu, 1989b). Airborne ascospores are considered more effective in initiating widespread epidemics because of their long range dispersal, higher inoculum potential and relative persistence within the canopy. The wide spread and sporadic nature of *Sclerotinia* rot of carrot epidemics also suggests that in carrot crops, ascospores are more likely to be the most significant primary inoculum. However, there are several common attributes that characterize both modes of disease initiation regardless of the primary inoculum; (i) sclerotia located near the soil surface are the most important source of inoculum for both infections;

(ii) both inocula need free moisture and an exogenous nutrient source to be infective; (iii) both modes require the presence of older or senescing leaves lodged on the soil surface and (iv) disease progress in both modes is encouraged by humid microclimate conditions within the enclosed canopy of mature carrot crops. These attributes are important considerations to address when attempting to design strategies for the management of *Sclerotinia* rot of carrot.

Regardless of the type of primary inoculum root infection results from infected foliage and occurs via the crown (Finlayson et al., 1989; Geary, 1978; Lewis and Garrod, 1983). Direct infection of carrot roots by mycelium arising from sclerotia in soil has not been observed and root infection always occurs after the foliage and crown become infected. Mycelium arising from lesions on foliage can progress through the petiole towards the crown form which it enters the root (Finlayson et al., 1989). Infection of carrot roots via petioles wounded by *S. sclerotiorum* contaminated machinery during mechanical harvest may be an additional source of disease in storage (Tahvonen, 1985). Following the crown pathway mycelium can circumvent the periderm which may be a structural barrier to penetration from the exterior of the root (Garrod and Lewis, 1982).

Optimum conditions for infection of carrots are prolonged periods of high moisture and temperature of 13–18°C (Rubatzky et al., 1999). Once infection is established, invaded tissues usually provide sufficient moisture for fungal growth and lesions expansion, however, prolonged dry weather conditions can suppress disease progress (Geary, 1978). Mycelium originating from diseased leaves can readily colonize adjacent senescing leaves, foliar debris lying on the soil surface and healthy foliage of neighbouring plants (Fig. 15.1). This is encouraged by the high plant density that is typical in current carrot cultivation practices. Therefore, secondary infection due to plant contact may be an important means of local spread of the pathogen that increases disease incidence in carrot crops. The pre-harvest cycle of *S. sclerotiorum* development is completed with the return to soil of new sclerotia produced on diseased leaves. These sclerotia will eventually germinate form apothecia or mycelium during subsequent seasons and initiate new epidemics of *Sclerotinia* rot of carrot.

16.9.2 The Post-harvest Epidemic

Disease of carrots in storage or in transit is a direct consequence of foliar and crown infection in the field (Finlayson et al., 1989; Geary, 1978, Lewis and Garrod, 1983) and every infected root has the potential to develop into a source of inoculum for new infections during storage (Fig. 15.1). However, incidence of foliar disease in the field may not quality as a good indicator of initial root disease incidence or potential crop loss in storage as no quantitative correlations between these cycles have been observed (Geary, 1978). The development of this secondary epidemic is a unique feature that characterizes *Sclerotinia* rot of carrot from many other *Sclerotinia* diseases but it becomes important when harvested carrots are designated

for storage or long distance transportation. Infections in storage are initiated mainly by mycelium arising from the crowns of diseased roots introduced form the field (Geary, 1978). The colonized root can provide sufficient nutrient reserves for the fungus to spread rapidly towards adjacent roots (Geary, 1978; Mukula, 1957). The presence of wounded tissues caused by mechanical harvesting or handling can increase susceptibility of carrots and encourage secondary infection in storage but is not essential (Geary, 1978). Mycelium of *S. sclerotiorum* persisting on the surface of infested wooden containers (Mukula, 1957; Rader, 1952; Subbarao, 2002) or in foliar debris adhering to the roots (Geary, 1978) may represent an additional source of contamination for carrots in storage.

Pre-harvest and post-harvest epidemics of *Sclerotinia* rot of carrot differ in several biological and environmental characteristics. Compared to foliar substrates, carrot roots are storage organs with higher nutrient content that encourage more extensive fungal growth and abundant sclerotia production. Humidity within storage is usually sufficient to favour fungal growth but low temperatures can substantially limit the development of *Sclerotinia* rot of carrot in storage. *S. sclerotiorum* can infect carrot roots in temperatures ranges from 0°C to 28°C, with maximum decay occurring at 23°C (Lauritzen, 1932; Mukula, 1957). Finally excluding sclerotia return to the field through manure fertilization, most new sclerotia produced on diseased roots in storage do not contribute to the increase of inoculum in soil and do not sustain the initiation of new epidemics, thus, although economically important this post-harvest cycle in the development of *S. sclerotiorum* is epidemiologically incomplete.

Chapter 17
Disease Forecasting

Diseases caused by *Sclerotinia sclerotiorum* and *Sclerotinia minor* are responsible for economically important losses on several crops including canola, cabbage, carrots, celery, lettuce, snap beans, soybean and white beans. Crops such as cabbage and celery, the etiology of these diseases are known, but little information is available on the epidemiology. In these crops, disease avoidance and cultural practices are the primary methods of disease management, although fungicides are sometimes applied after symptoms are observed. For other crops such as beans, canola, carrots, lettuce and soybean, the epidemiology has been described and at least partially quantified. Based on these epidemiological studies, disease-forecasting systems have been developed for canola, lettuce and beans and another is currently being developed for carrots. Epidemics in snap beans are associated with ascospores infecting petals as the primary inoculum and forecasting is based on soil moisture, rainfall, crop flowering, canopy enclosure and apothecia. Epidemics in carrots are bicyclic and represent a different situation. Epidemics in the field are associated with infection on senescing leaves in contact with moist soil under the carrot canopy. Forecasting is based on soil moisture, canopy enclosure, senescing leaves, air and soil temperature and the presence and number of apothecia. Epidemics in storage are associated with air temperature, rate of cooling, surface wetness and pre-existing infection. Despite the availability of forecasting systems for diseases caused by *Sclerotinia* spp. on several crops, there are no examples of organized monitoring or forecasting programs for these diseases. Anecdotal comments suggest that the reasons for the lack of development and implementation of forecasting models include the variable severity of epidemics, a lack of registered fungicides, little or no infrastructure to deliver disease-forecasting systems, and declining prevalence of integrated pest management (IPM) programs (Mc Donald and Boland, 2004). However, information has been generated on forecasting of following *Sclerotinia* diseases.

G.S. Saharan, Naresh Mehta, *Sclerotinia Diseases of Crop Plants: Biology, Ecology and Disease Management*,

17.1 *Sclerotinia* Stem Rot of Rapeseed

The need for forecasting *Sclerotinia* stem rot of rapeseed has been recognized in countries such as Denmark, Germany and Canada. The possibility of forecasting stem rot of rapeseed based on petal infestation (PI) with the pathogen was first suggested by Gugel and Morrall (1986) and later refined by Turkington et al. (1991a). In a study of inoculum disease relationships, a strong relationship between disease incidence and percentage PI at early bloom stage has been established. Sampling at five to six sites per crop and plating 40 petals per site is enough to estimate percentage PI with standard error of about 5 per cent in most fields. It is good to collect petals in the afternoon and should wait several hours after a rainfall as precaution against slight under estimation of PI values. Canopy density affects stem rot. By altering the microclimate in the crop, the relationship between inoculum and disease incidence is also affected. More disease occurs per unit of PI in dense crops (Turkington et al., 1991b). Kamensky (1987) developed and tested a kit which included agar plates, forceps, disinfectant and written and video taped instructions for sampling petals and setting up a test. Thomson and Morrall (1991) prepared a manual containing colour photographs illustrating the difference between *S. sclerotiorum* and common saprophytes which develop from rapeseed petals. Using these photographs and a key in the manual, about 45 growers from across western Canada successfully used a modified kit to set up petal tests and read the results. The manual used by growers in 1990 has been modified to correct minor problems that occurred in its use, but it is clear that growers can successfully use a kit to conduct their own petal tests. It is recommended that growers consider upto three successive petal tests during flowering to account for fluctuations in PI, but unless PI remains low, only two are usually necessary. Petal testing has several advantages over other methods of forecasting stem rot of rapeseed. It is applied on an individual crop basis. It is superior to searching for apothecia in accounting for sources of inoculum that are aggregated or extrinsic to the crop. Finally, in the disease cycle, infested petals are a few steps closer than apothecia to the forecast target, namely diseased plants, thus, there is less potential for environmental intervention between forecast and reality. However, petal testing will never prevent unnecessary fungicide applications when a high disease risk is not translated into high disease incidence because of dry weather after flowering. This deficiency applies to all forecasting systems for *Sclerotinia* stem rot. However, according to Bom and Boland (2000), the model that includes petal infestation and soil moisture predicts more fields correctly than the model using petal infestation alone, but the accuracy of both is affected by the timing of soil moisture measurements in relation to petal infestation and threshold values in discriminating categories of soil moisture and petal infestation. Twengstrom et al. (1998a) suggested a forecasting system of *Sclerotinia* stem rot in spring sown oilseed rape.

A simple forecasting system with fairly good reproducibility for evaluation of the risk of attacks of *Sclerotinia* in rape has been developed in Denmark (Buchwald, 1986). The negative forecasts which advise against chemical control have turned

out to be most reliable, while the calculation of the positive risk of attacks is built on the following experience and test results gathered in the last five years. The forecasts are based on the following information's:

(a) Accumulated number of germinated sclerotia in depots, including the number of sclerotia with active apothecia (turgid, light brown).
(b) The frequency of apothecium occurrence in rape fields selected at random and in fields with previous attack of *S. sclerotiorum*.
(c) The growth stage of the oilseed rape as compared with the development of the fungus.
(d) Rainfall (and temperature) at localities with depots of sclerotia.
(e) Weather prognosis for five days at the time of the forecast.
(f) High apothecial development only takes place after a rainfall of a minimum of 30 mm within a period of 7–14 days. On the other hand, this precipitation does not necessarily cause a high germination because of evaporation or an unfavourable microclimate.
(g) To cause any serious damage, the germination of the sclerotia must have started 7–14 days before initial flowering (Growth stage 4.1). Apothecia formed after this time will come too late to do any damage.
(h) Preliminary experience seems to indicate that there is a risk of attacks when the accumulated number of germinating sclerotia in the depots is over 30 per cent at the time of the forecast. Besides this, the majority must have active apothecia.
(i) After a rainfall of a minimum of 30 mm, naturally occurring apothecia can be found within a period of 7–14 days, especially in fields with previous attacks of *S. sclerotiorum*.
(j) During the week after a rainfall of a minimum of 30 mm apothecia are formed and become visible.
(k) One week without rainfall prevents or delays the formation of new apothecia and dries out those.

17.2 *Sclerotinia* Stem Rot of Soybean

A correlation model between disease incidence and weather factors has been established from records over five years in Heilongjiang Province, China by Chenz and Jiao (1994). Stepwise multiple regression calculations provided an equation by which the severity of the disease epidemics can be predicted. A good fit is found between observed and theoretical values. An exponential regression equation has been developed to predict epidemics using the number of apothecia during the blossom stage. Thus, both mid and short term disease predictions can be made. In China, model of relationship between the per cent of yield loss (Y) and the quantity of apothecia (X) of *Sclerotinia* rot of soybean has been established ($Y = -4.5499 + 2.313X$; $r^2 = 0.8442$). The disease control threshold is three to four apothecia per $9.75\,m^2$ (Pan-Hong et al., 2001).

17.3 *Sclerotinia* Disease of Lettuce

The feasibility of developing a forecasting system for carpogenic germination of *Sclerotinia sclerotiorum* sclerotia has been investigated in the laboratory by determining key relationships among temperature, soil water potential and carpogenic germination for sclerotia of two *S. sclerotiorum* isolates. Germination of multiple burials of sclerotia to produce apothecia also has been assessed in the field with concurrent recording of environmental data to examine patterns of germination under different fluctuating conditions. Carpogenic germination of sclerotia occurs between 5°C and 25°C but only for soil water potentials of < more or = >−100 k Pa for both *S. sclerotiorum* isolates. Little or no germination occurs at 26°C or 29°C. At optimum temperatures of 15–20°C, sclerotia buried in soil and placed in illuminated growth cabinet's produces stipes after 20 to 27 days and apothecia after 27 to 34 days. Temperature, therefore, has a significant effect on both the rate of germination of sclerotia and the final number germinated. Rate of germination is correlated positively with temperature and final number of sclerotia germinated is related to temperature according to a probit model. Thermal time analysis of field data with constraints for temperature and water potential shows that the mean degree days to 10 per cent germination of sclerotia is 285 and 279, respectively and generally is a good predictor of the observed appearance of apothecia. Neither thermal time, nor relationships established in the laboratory can account for a decline in final percentage of germination for sclerotia buried from mid-May compared with earlier burials. Exposure to high temperatures may explain this effect. The present and other factors, require investigation before relationships is derived in the laboratory or thermal time can be incorporated into a forecasting system for carpogenic germination (Clarkson et al., 2004a, b).

17.4 *Sclerotinia* Blight of Peanut

Algorithms have been evaluated for computing disease risk and improving the timing of fungicide applications for the control of *Sclerotinia* blight (*Sclerotinia minor*) of groundnut. Disease risk is calculated by multiplyig indices of moisture, soil temperature, vine growth and canopy density each day and summing values for the previous five days to obtain a five days Field Disease Risk Index (FDRI). After fungicide application, the FDRI is reset to zero for three weeks. Fluazinam at 0.58 kg a.i./ha applied at FDRI 24 or 32 suppresses disease and increases yield as much as or more than programmes of weekly scouting and applying fungicide at the initial onset of disease with additional sprays at three to four week intervals. The FDRI algorithm is also more efficient than calendar sprays at 60, 90 and 120 days after planting (DAP). These parameters, along with DAP-dependent thresholds, consistently improves the timing of fungicide sprays and disease management

when using the FDRI algorithm compared to weekly scouting or calendar sprays at 60, 90 and 120 DAP (Langston et al., 2002).

17.5 White Mold of Snap Bean

The occurrence of ascospores and incidence of white mold in snap bean crops have been predicted using soil matric potential thresholds (Hunter et al., 1984).

Chapter 18
Disease Resistance

18.1 Biotechnology

Biotechnological approaches to enhance disease resistance involves either exploitation of natural forms of resistance or genetic engineering approaches, such as the introduction of chitinases, glucanases and other antifungal proteins. The former approach may involve the introduction of novel resistance genes from wild species and the subsequent introgression of genes through the use of molecular markers, or attempts to clone resistance genes. The approach to gene cloning which is most likely to be successful to exploit *Arabidopsis*. Chitinases, which hydrolyse the β-(1-4)-glycoside in chitin (a major component of fungal cell walls), are often induced in plants following fungal attack and it is thought that these are involved in plant defense. Lines which contain chitinases that are specifically induced in *Brassica* by pathogens may give a higher degree of protection. Another strategy has been the introduction of a gene for oxalate oxidase in order to reduce susceptibility to infection by *Sclerotinia sclerotiorum* which relies upon the production of oxalic acid in the infection process.

18.1.1 Development of Transgenics

Field resistance to *S. sclerotiorum* in some crops has been correlated with laboratory resistance to oxalic acid (Kolkman and Kelly, 2000; Wegulo et al., 1998). A defense strategy against *S. sclerotiorum* in crop species is the use of transgenes that specially degrade oxalic acid produced by *S. sclerotiorum*. The wheat germin gene coding for an oxalate oxidase catalyses oxidation of oxalic acid by molecular oxygen to CO_2 and H_2O_2 (Lane et al., 1993). H_2O_2 is also generated from the oxidative burst and has been implicated as an important factor in the plant HR (Levine et al., 1994; Wojtaszek, 1997). Further more, H_2O_2 may be directly toxic to microbes (Peng and Kuc, 1992) or may result in salicylic acid accumulation, an important signaling molecule in systemic acquired resistance (Gaffney et al., 1993; Leon et al., 1995). Therefore, transgenic oxalate oxidase in crop species

G.S. Saharan, Naresh Mehta, *Sclerotinia Diseases of Crop Plants: Biology, Ecology and Disease Management*,

might have indirect beneficial properties besides degradation of oxalic acid. Oxalate oxidase and other oxalic acid degrading enzymes have been incorporated into several important crops such as soybean, sunflower and peanut, which have shown increased resistance to *Sclerotinia* spp. (Donaldson et al., 2001; Hu et al., 2003; Kesarwani et al., 2000; Livingstone et al., 2005). One of the disadvantages to this technology is the potential escape of transgenes into wild plants, possibly leading to more invasive wild species. A study on the environmental impact of oxalate oxidase transgenes escaping from cultivated sunflower into wild relatives revealed that oxalate oxidase does contribute to enhanced *S. sclerotiorum* resistance when backcrossed into wild sunflower. However, because the transgenes did not significantly affect seed production and reproductive out put, the contribution of these transgenes did not give a fitness advantage to the new host plants, which suggests that oxalate oxidase may diffuse neutrally after apotential escape (Burke and Rieseberg, 2003). Transgenic lettuce lines containing the decarboxylase gene (oxdc) from a *Flammulina* sp. have been produced by *Agrobacterium* mediated transformation (Dias et al., 2006).

18.2 Mechanisms of Host Resistance

It is probably not reasonable to expect pronounced resistance against a fungus with such a wide host range within one of its host species or even a genus. In addition, strain specificity in regard to pathogenicity to various hosts has not been reported. The dearth of reports before 1968 indicates that many researchers formerly accepted the idea that resistance to *S. sclerotiorum* does not exist. In earlier studies, field resistance to *S. minor* has been observed in red and white clover (Aldrich, 1974) and alfalfa (Elgin and Beyer, 1968). Escape from *S. sclerotiorum* infection due to type of growth habit has been reported in lettuce (Newton and Sequeira, 1972b), sunflower (Laclerca, 1973) and beans. Differences in susceptibility of cvs. breeding lines and plant introductions are noted in soybean (Grau and Bissonnette, 1974), peanut (Porter et al., 1975), and sunflower (Orellana, 1975). Orellana (1975) attributed tolerance of sunflower to enhanced growth and lignification of host tissues in response to long-day treatment. Kanbe et al. (1997a) suggested that the growth of *S. sclerotiorum* hyphae invading resistant strains of alfalfa is inhibited due to browning of the host cells.

The physiology of *Sclerotinia* disease resistance has not been studied adequately and in fact, disease resistance among many susceptible genera of dicotyledonous plants has not been found. Monocots generally are immune or very resistant (Lumsden, 1979). Three general types of resistance to *Sclerotinia* spp. have been observed. First resistance of tissue to breakdown is possibly associated with nutrition of the fungus, second presence of preformed antifungal materials and third formation of phytoalexins.

18.2.1 Beans

Resistance of scarlet runner bean to *S. sclerotiorum* appears to be due to in part at-least to a physical barrier to infection or middle lamellae of host cells that greatly impede penetration and infection. The resistance in *Phaseolus coccineus* is characterized by limitation of lesion size and the formation of small brown lesions. Histological examination shows that several stages of infection differ strikingly from susceptible, *P. vulgaris* infection. The differences in the resistant host include: (i) penetration of the cuticle of *P. coccineus* often is impeded. (ii) Secondary infection cushions often develop beneath the cuticle and adjacent to the epidermis and (iii) infection hyphae in *P. coccineus* are often small, distorted and not subcuticular. These differences suggest that the resistant tissue acts as a physical barrier or the middle lamella is not readily degraded to allow rapid penetration and infection (Dow and Lumsden, 1975). The growth of *S. sclerotiorum* in white beans is much slower in the tolerant cv. Ex Rico-23 than in the susceptible cvs. Kentwood and Seafarer. The difference is paralleled by the rate of diffusion in leaf tissue of oxalic acid, a phytotoxin of the white mould fungus. Uptake of oxalic acid into excised leaves through the petiole results in more severe brown rot like symptoms in the leaves of cv. Kentwood than in those of Ex Rico-23. When [14 C] oxalic acid is fed through petioles, radioactivity in the inter veinal tissue of Kentwood is about three times higher than that in Ex Rico-23. Autoradiograph of leaves shows that radioactivity in Ex Rico-23 is confined to major veins and that activity in the interveinal tissue is low, whereas in Seafarer and Kentwood radioactivity does not accumulate in the veins but is distributed uniformly throughout the leaf tissue with a slight accumulation along the leaf margin (Tu, 1985). Phillips et al. (1993) investigated the partial physiological resistance (PPR) of common beans to the white mould pathogen. The activity of phenylalanine ammonia-lyase (PAL) has been measured in five bean cvs. viz., Upland, Benezi, Sierra, UI-114, and Montcalm and one breeding line, NY-5394. All cvs. varied in PPR to white mould disease. Greater PAL activity in the resistant NY-5394 than in the susceptible Upland suggests that PAL activity may be involved in the PPR of common beans to *S. sclerotiorum*.

Coyne et al. (1976) observed a low level of white mould in the small white dry bean"Aurora." This variety has a porous canopy and possesses plant architectural features like upright determinate or short indeterminate habits, consisting of a few main stems internodes, few short side branches and small trifoliolates. These plant habits should facilitate improved air circulation and better light penetration within the canopy resulting in a more rapid drying of dew covered leaf surfaces which may contribute an avoidance mechanism to reduce white mould disease. According to Saindon et al. (1995), upright bean cvs. can be grown at high planting densities (25–60 plants per square meters) without greatly increasing the risk of a white mould outbreak.

Genetic blends are not successful in protecting the indeterminate susceptible GN Nebraska #1 against high white mould infection under a moderate or severe level of white mould incidence (Coyne et al., 1978). A breeding progamme to combine genetic resistance and architectural disease avoidance to white mould (Coyne et al.,

1976) along with microclimate modification due to cultural practices (Blad and Steadman, 1975) offers a much better prospect how genetic blends of achieving a practical and economic level of control of white mould disease in indeterminate dry bean cvs. The resistant cvs. of French bean have the lowest content of amino acids of acidic group (aspartic and glutamic acids) and the highest content of those of the alkaline and aromatic groups in the root exudates and leaf proteins. Resistance cvs are also having the lowest content of sugars and highest content of cellulose and hemicellulose in the leaves. The presence of amino acids of the aromatic and alkaline groups in the root exudates inhibits fungal growth (Pieta, 1990, 1994). Cruickshank and Perrin (1971) suggested the role of phaseollin in resistant bean cvs.

18.2.2 Clover

Resistance of clover to *S. trifoliorum* has been postulated to be due to more efficient use of food reserves by certain clover varieties, resulting in a resistant middle lamella that is less easily hydrolyzed by enzymatic action (Held, 1955). In addition, failure of non-hosts to induce infection hyphae formation suggests a nutritional basis for resistance (Lumsden, 1975).

Preformed antifungal materials have been examined in clover leaves infected with *S. trifoliorum* (Debnam and Smith, 1976). Several isoflavones released from glycosidic combination on infection exhibit little activity towards *S. trifoliorum*. Antifungal activity towards *S. trifoliorum* has been shown for 7-hydroxy-4-methoxy-isoflavone from clover (Virtanen and Hietala, 1958). Clover cvs. resistant to *S. trifoliorum* consistently accumulate more phytoalexin than susceptible ones, but the final overall concentration in the resistant cvs. would not have inhibited *S. trifoliorum in vitro* (Debnam and Smith, 1976).

18.2.3 Celery

There are evidences that (+) marmesin, rather than linear fluranocoumarines (psoralins) may play the major role in celery resistance to pathogens during storage. (+) marmesin, the precursor of psoralins in celery has at least 100 times greater antifungal activity *in vitro* in the dark than psoralins. An increase in celery decay is negatively correlated with (+) marmesin concentration and positively correlated with psoralin concentration (Afek et al., 1996).

18.2.4 Sunflower

Helianthus resinosus possesses pre-existent defense mechanisms like cortical sclerified fibre cells, *sclerenchyma*, numerous and voluminous glandular hairs, epidermic flavonoids and caffeo lquinic compounds which provide multiple mechanisms of resistance

against *S. sclerotiorum* in this wild species (Mondolot-Cosson et al., 1994). A 5 Kda antifungal peptide (AP5) has been isolated from *H. annuus* (line HA89) leaves infected with a virulent isolate of *S. sclerotiorum*. The peptide *in vitro* inhibits ascospore germination of the fungal pathogen *S. sclerotiorum* and produces mycelial growth inhibition @ 0.4 m μM (Regente et al., 1997). Resistance in SDP AC-l is associated with high quantity of phenolic compounds (Hemery-Tardin et al., 1998). However, according to Moley et al. (1990) tolerance is associated with an earlier and greater accumulation of RNA and use of hydroxyproline rich glycoprotein RNA as a molecular marker is possible. Hemery-Tardin et al. (1998) suggested that phenolic compounds in healthy sunflower plants can be used as markers of *Sclerotinia* resistance. Higher constitutive and induced phenolic content as well as phenylalanine ammonia lyase activity are present in the most resistant lines of sunflower against *Sclerotinia* (Prats et al., 2003).

18.2.5 Vegetables

Preformed materials have been found to be associated with resistance of onion and potato to *S. sclerotiorum* infection (Echandi and Walker, 1957). Unidentified substances from resistant potato tissue inhibit maceration of susceptible radish, cucumber and carrot tissues by extracts from *S. sclerotiorum* cultures. Immune onion tissue extracts completely prevent maceration.

18.2.6 Rapeseed-Mustard

In resistant cvs. of *B. juncea*, in diseased stems, phenolics accumulate at the infection site and there is a relatively low level of enzyme activity compared to that in the susceptible cv. (Rai et al., 1979). According to Tewari and Conn (1992), the pathogenesis of *S. sclerotiorum* in rapeseed reduces due to sequestration of oxalic acid by calcium. If enough calcium is applied to chemically tie up all the oxalic acid produced by *S. sclerotiorum*, infection is not likely take place.

Constitutive over expression of a protein involved in plant defense mechanisms to disease is one of the strategies proposed to increase plant tolerance to fungal pathogens. A hybrid endochitinase gene under a constitutive promotor has been introduced by *Agrobacterium* mediated transformation into a winter-type oilseed rape (*B. napus* var. *oleifera*) inbred line. When progeny from transformed plants are challenged by pathogens, plants exhibit an increased tolerance to disease as compared with the nontransgenic parental plants (Grison et al., 1996).

18.2.7 Carrot

Polyacetylenic compounds possess strong antifungal properties and are associated with physiological resistance of carrots. The concentration of falcarindiol in the

young root periderm is negatively correlated with the susceptibility of cultivars to *S. sclerotiorum*, suggesting its role in providing resistance of carrots to *Sclerotinia* rot of carrots in storage (Olsson and Svensson, 1996). Ontogenic (age related) resistance is expressed in young and active leaves that are shown to react hypersensitively to the penetration of *S. sclerotiorum* (Geary, 1978).

18.3 Genetics of Host-Pathogen Relationship

Marker assisted selection may aid in the research to find and incorporate physiological resistance into crop cultivars. Markers tightly linked to resistance genes and quantitative trait loci (QTL) allow screening on the basis of genotype as well as phenotype, maximizing the effectiveness of selection. Kim and Diers (2000) found three QTLs for *S. sclerotiorum* resistance in soybean. Two out of three of these loci are associated with escape mechanisms. However, the third QTL is not linked to any escape mechanisms suggesting it may contribute to physiological resistance to the disease. QTLs for resistance to the diseases caused by *S. sclerotiorum* have been reported in several other important crops such as sunflower, common bean and oilseed rape (Arahana et al., 2001; Bert et al., 2004; Kolkman and Kelly, 2003; Miklas et al., 2003; Zhao and Meng, 2003a, b).

18.3.1 Beans

Inheritance of resistance in beans to *S. sclerotiorum* has been studied in Nebraska (Coyne et al., 1977a) and New York (Abawi et al., 1978). In *Phaseolus vulgaris* crosses of resistant Black Turtle Soup × Great Northern cvs. and lines, heritability of the disease reaction is low. The limited populations of *P. vulgaris* × *P. coccineus*, in B-3749 resistance appeared to be controlled by a single dominant gene (Abawi et al., 1978). Abawi et al. (1978) was able to transfer resistance to *S. sclerotiorum* through several backcross generations in snapbean (*P. vulgaris*). Lyons et al. (1987) indicated that recurrent selection may be a useful technique for the development of resistance to white mould in *Phaseolus* spp.

Inheritance of resistance to *Sclerotinia* stem rot in faba bean (*Vicia faba* L.) is controlled by a single dominant gene (Lithourgidis et al., 2005). Miklas et al. (2001) identified three QTL loci from two resistant sources that condition physiological resistance.

18.3.2 Cabbage and Cauliflower

Resistance to stalk rot has been found to be polygenic under the control of recessive genes and due primarily to additive gene action. Progeny of the cross EWAW × Janavon has the lowest stalk rot incidence (Baswana et al., 1991). However, according

to Dickson et al. (1996) cabbage and cauliflower resistance against *S. sclerotiorum* is governed by a major recessive gene plus modifiers.

18.3.3 Sunflower

Interspecific hybrids of *H. tuberosus* × *H. annuus*, and *H. tuberosus* × *H. strumosus* are reported to be resistant to stalk rot (Orellana, 1975). Pirvu et al. (1985) reported monogenic resistance with a recessive gene in the lines, CS-77-999-1 and CS77-1081 responsible for the mechanical resistance of the outer tissues that protect the stalk. According to Rashid and Dedio (1992), resistance to stalk rot appears to be conditioned by additive genes derived from both parents. The resistant clones can be further propagated to act as source for protoplasts providing disease resistance to *H. annuus* cvs. via somatic hybridization (Henn et al., 1997). Eight QTLs have been detected for resistance to mycelial extension in sunflower plant tissue. Four QTLs explains 65 per cent of the genetic variance for the speed of the growths of *S. sclerotiorum* in leaf and petal tissue (Hahn et al., 2001). Resistance to *S. sclerotiorum* in sunflower is governed by a considerable number of QTLs, located in almost all the sunflower linkage groups (Bert et al., 2004).

Using the composite interval procedure, Ronicke et al. (2005) identified three QTLs' for lesion length and two QTLs' for head rot resistance to *S. sclerotiorum*. Overall, the QTLs' account for 60 per cent of the genetic variation for leaf resistance and 38 per cent for capitulum resistance to *S. sclerotiorum* (Mestries et al., 1998).

18.3.4 Peanut

In peanut resistance to *Sclerotinia* blight appears to be quantitatively inherited in addition to the cytoplasmic factors as indicated in crosses with Chico and Florigiant (Coffelt et al., 1980). However, later Coffelt and Porter (1982) reported morphological and physiologic resistance to *Sclerotinia* wilted peanuts. The physiologic resistance is partially controlled by a cytoplasmic factor. According to Wildman et al. (1992), inheritance of resistance in peanut to *S. minor* is complex.

18.3.5 Rapeseed-Mustard

The heritability of *Sclerotinia* resistance is high in *B. napus*, controlled by nuclear genes and unlinked to the low erucic acid trait. An apetalous mutant of *B. napus* is substantially free of stem rot compared to the normal petalous cv. Wester (Liu et al., 1990). The inheritance of *S. sclerotiorum* resistance in *B. napus* is partially dominant (Huang-Yong Ju et al., 2000). Fu et al. (1990) studied the inheritance of

the apetalous character in *B. napus* and showed that four recessive genes control this trait. The apetalous lines are unaffected by stem rot. Genetic analysis of resistance to *S. sclerotiorum* in *B. napus*, 15 days after inoculation to petals is controlled by major genes with additive dominant epistatic effects as well as by polygenes with additive dominant epistatic effects (He Kun Yan et al., 2005). According to Zhao and Meng (2003a), both single locus quantitative trait loci (QTL) and epistatic interactions play important roles in *Sclerotinia* resistance in rapeseed. A new more sensitive method for heritability detection for resistance to *S. sclerotiorum* lesion expansion in rapeseed (*B. napus*) has been suggested by Liu Sheng Yi et al. (2003). In *B. napus*, one of the nine loci associated with aliphatic glucosinolate content is associated with *Sclerotinia* resistance (Zhao and Meng, 2003b).

18.3.6 Soybean

Twenty eight putative QTLs for resistance to *Sclerotinia* stem rot of soybean have been identified on 15 different linkage groups in five recombinant inbred lines (Corseoy 79, Dassel, DSR 173, S19–90, Vinton 81 exhibiting partial resistance) populations. Seven QTLs on seven different linkage groups have also been identified in multiple populations with some QTL regions corresponding with mapped resistance genes and resistance gene analogues. Several genes control resistance to *Sclerotinia* stem rot and markers can facilitate an initial screen of segregating breeding populations (Arahana et al., 2001).

18.3.7 Alfalfa

The effect of selection on *Sclerotinia* crown and stem rot (SCR) resistance increases in later generations, probably due to the accumulation of genes with minor or polygenic effects on resistance (Kanbe et al., 1997).

18.4 Induced Resistance

Salicylic acid (SA), l-amino-cyclopropane-l-carboxylic acid (ACC) and DL-beta-amino-n-butyric acid (BABA) have been screened for the ability to induce phenylalanine ammonia lyase (PAL) activity in kiwifruit (*Actinidia deliciosa* cv Hayward) leaves. SA (2 mM) is the most effective and induces a ten-fold rise in PAL activity after two days compared with a four fold rise five days after ACC (0.05 mM) treatment. BABA is not an effective elicitor of PAL. SA has been further tested, alongside a chlorinated analogue –4-chlorosalicylic acid (4CSA) for the ability to control *S. sclerotiorum* on kiwifruit leaves. Pre-treatment with SA and 4CSA causes

a reduction in the size of lesions arising from subsequent *S. sclerotiorum* infection. 4CSA is the more effective and reduces disease levels, relative to controls by up to 85 per cent on leaf discs and 78 per cent on leaves on the vine. This compares with a 48 per cent reduction by SA on both. Resistance to infection is not affected by washing treated leaf discs prior to inoculation or by delaying inoculation for up to four days following 4CSA application. SA and 4CSA are rapidly absorbed and metabolized by kiwifruit leaves and have no apparent phytotoxic effects at the concentrations used for disease control studies. It is proposed that SA and 4CSA operate through the induction of host resistance mechanisms (Reglinski et al., 1997). Induction of local and systemic resistance in tomato and cauliflower by spray of *Trichoderma harzianum* and *T. viride* against stalk rot caused by *S. sclerotiorum* has been observed (Elad, 2000; Sharma and Sain, 2004). Chitosan induces resistance against *S. sclerotiorum* in carrots treated with enzymatically hydrolysed chitosan (Molloy et al., 2004). Oxalate oxidase can confer enhanced resistance to *Sclerotinia* blight in peanut (Livingstone et al., 2005). Application of herbicides Lactofen and Action induces resistance to control stem rot of soybean (Yang and Lundeen, 2001). INA or BTH induces resistance in soybean (Dann et al., 1998). In soybean, Glyphosate resistant cvs. S20-B9 and 93 Bol produce more phytoalexin than glyphosate susceptible cvs. S-19-90 and P2g1 (Nelson et al., 2002a). When oxalic acid is applied to a discrete area of oilseed rape leaf, significant local resistance is expressed in the surrounding leaf tissue and maximum resistance being exhibited by the tissue closest to the site of petiole attachment (Toal and Jones, 1999).

Non-ionizing ultraviolet (UV-C) radiation can effectively elicit the accumulation of the antifungal phytoalexins 6-methoxymellein (6 mm) in carrot roots and hence, induce systemic resistance to subsequent infections by *S. sclerotiorum* (Mercier et al., 1993). Treatment with UV-C radiation at a dose of 2.20×10^5 erg cm^{-2} induces accumulation of 6 mm to maximal inhibitory levels (e.g., 60 g^{-1}) and reduces at 1°C or 4°C. However, integration of UV treatments with other control strategies is recommended for a prolonged protective effect (El Ghaouth, 1994).

18.5 Sources of Resistance

Due to sporadic nature of disease outbreaks, especially for ascospores initiated diseases as they are highly dependent on environmental conditions, screening for resistance under field conditions is often problematic without irrigation and artificial inoculation. Furthermore, it is not known which portion of the resistance in the field is the result of physiological resistance or escape mechanisms such as flowering date, lodging, canopy architecture and maturity, which have all been associated with disease severity (Boland and Hall, 1987; Kim and Diers, 2000; Nelson et al., 1991a). Therefore, greenhouse and laboratory screening is often an integral part of resistance screening methodology (Dickson et al., 1996; Kim et al., 2000; Miklas et al., 1999; Whipps et al., 2002; Zhao et al., 2004). The various sources of resistance in different crops are presented in Table 18.5.1.

Table 18.5.1 Sources of resistance in different crops against *Sclerotinia*

Crop	Resistant sources
Beans	GN-Nebraska, Black Turtle Soup, Sanilac, Capital, Dark Red Kidney, Aurora, Charlevoix, Valentines, Venzuela-350, Soldier, Steuben, Yellow eye, NY-69-6207-2, Rico-23, Ex Rico-23,Crestwood, Centralia, Cvs-A-51, 83, VEF MXA-222, PI-169787, PI-175829, PI-189567 (*Phaseolus coccineus*), PI-226865, PI-263936, Biola, Wyborowa, Wiejska, Saxobel, IGE-1179, IGE-2179, NY-5223, NY-5268, PER-257, Bat-447-IC, Bac-17, Xan-170, A-480, Pad-22, Flair, Pearly, Wonji, Shimi, Vribe, Redondo, AC Skipper, Cvs-VI-911, Cvs-VI-137, Dunav 1, Padej 1, Isobella, A 195, NAB 19, IIPR 7585 and SIN 11
Lettuce	Gallega d' hiver, Blondea bord rouge, Great Lakes-54, Tetue de Nines, COS, *Lactuca dentata*, *L. perennis* and *L. serrima*
Cauliflower	Janavon, Super Snowball, Sel-12, Early Winter, Adam's White head, EC-162587, EC-173803, EC-173807, EC-177283, RSK-1301, MKS-1, EC-103576 and EWAWH
Soybean	Corsoy, Hodgson, Hodgson-78, Maple Arrow, Maple Ridge, Maple Presto, Ace, Partage, McCall, Ozzie, Pella, Carsoy-79, Hardin, Plamya, Grant, Dong Nong-37, Dong Nong-39, Dong Nong-30, Abyara, AXN 1–55, PI 153, PI 282, PI 189, PI 931, PI 196, PI 157, PI 398, PI 637, PI 417, PI 201, PI 818, Mandarin (Ottawa), PI 248509B, PI 384942, PI 423853, PI 503336, PI 504497, PI 507327, PI 578496, PI 592949, PI 592953, PI 594286, S19-90, NKS 1990, Asgrow, A 2506 and Colfax
Safflower	AC Sunset, RHA 439, RHA 440 and HA 441
Linseed	Antares and Narlin
Peas	PI-155109, PI-166188, PI-189171, PI-261622, PI-262189. PI-263027, PI-272191, PI-272205, PI-272209, ID-89-1, ID-2, Dark Skin Perfection, Perfection-132, Wisconsin Perfection, DPP-19, DPP-54, 5 DPP-8, DPP-71, DPH-9, DPH-96, HPPC 95, P-3477, P-3496, P-3549, P-3611, P-3641 and P-3673
Brinjal	V-1198, V-1200, V-1687, V-1740 and V-1755
Alfalfa	Nicrzd, NCMP-2, MSR, Florida-5472, WL 414, WL 325, WK 323, CS 40A, CS 40R, LR43112-7, CRSY 541-1, CR 488-3, Everest, Derby, Lutece, Furez 507 and Delta
Clover	Suminskii, Mestnyl, Vazhskii, Mestnyl, Resident otofte, Kenster (4x), Marino (2x), P-35-5 (4x), P-28-3 (2x), P-28-5 (4x), Tepa (4x), Temera (4x), St-448 (4X), Tetri Lassam, Vanessa, Diper, Kuhn, Noc, Albatros, Jutin and Arimaicai
Peanut	Chico, NC-3033, Florigiant-17165, PI-343392, PI-371521, VA-71-347, NC-3033, VGP-1, Virginia-81, TX-804475, Toalson, TX-798731, TX-798623, TX-798736, TX-AG-4, TX-AG-5, VA 93B, Catelo, Kwarts, N 92056C, Tamrum 98 (TX 901417) and Perry (N93112C)
Sunflower	HA-61, Lovaszpatonia, GOR-104, Nagykarosi, CM-361, CM-953-8-3, CM-90RR, CM-497, CM-526x, CM-361, CM-953-8-3, EC-36328, Chernyanka, PK-04/75, Waldsspindel, Interen Violet LeRennes, Gorno-Altalskii, Vengreskii, S-254, Varonezhskii-523, Oderskii-103, Sunbred-254, Soldor-200, S-5991, S-6269, Russian-29, Ai-113, VIR-130, VIR-160, Sunstar 377, Pioneer 6480, Pioneer 6479, HA 390, RHA 391, RHA 392, RHA 408, RHA 409, RHA 410, HA 411, HA 412, HA 61, 61-1, Lavaszpatonia, GDR 104, Yugykorosi, P21, MSXHA 61, PK 104175, PI-377530 and PI-38057

(continued)

Table 18.5.1 (continued)

Crop	Resistant sources
Rapeseed-Mustard	Omi nature, Isuzu (*Brassica napus*), Hallayucke, Norin-9, Doral, Librador, Lirama, Bor, BOH-1592, BOH-1693, MAH-1391, MAH-1592, BOH 2600, HH-1, OKEG-8, OKEG-94, POH-285, H-43/33, Jet Neuf, Koganenatane, Aburamasari, Kizakinonatane, Cutton, ZYR6, PSM 169, PDM-1969, Wester, PYM 7, Parkland, Tobin, PCR 10, Candle, Cutlass, Torch, RW 8410, RW 9401, RGN 8006, Hyola 401, PBN 9501, PWR 9541, Kiran, RH 9401, RH 492, PAB 9511, Bermuda, Capio, Mohican, Bor, Xiangyou 15, Zhongchuang 9, MAH 1996, Valesca, Passat, Libro, BKH 894, PNG 2170, MA 1615-1, MZL 236, BK 2466/93 and MA 1649-1
Sweet potato	Beauregard
Dolichos bean	Arka vijay, 6009, 6022, 6802, 7011, 7020 B, 7101, 7202, 8101 and Rajani
Cucumber	Ganfeng 2, Zhongnong 2, CI and A 15

18.5.1 Beans

Sources of resistance to *S. sclerotiorum* in beans were first identified by Anton de Bary in 1887 when he found that *Phaseolus multiflorus* (*P. coccineus*) was seldom attacked whereas *P. vulgaris* (common bean) cvs. were destroyed by the fungus. Adams et al. (1973) confirmed that *P. coccineus* (scarlet runner bean) is resistant. Abawi et al. (1978) reported resistance in *P. coccineus* (PI-175829 from Turkey) and *P. coccineus* × *P. vulgaris* hybrids. The near isogenic determinate ITGN Nebraska #1 has lower white mould infection than the indeterminate GN Nebraska #1 (Coyne et al., 1978; Steadman et al., 1973). Dark Red Kidney and Charlevoix have low level of infection in a severe field test (Steadman et al., 1974). Hunter et al. (1981, 1982a) reported *P. coccineus* tender pod and *P. coccineus* sub. sp. *polyanthus* as resistant. An upright vineing habit contributes much to bean resistance cvs. Black Turtle Soup, Sanilae, Capitol, Aurora, Charlevoix, Valentine, Venezuela-35, Soldier, Steuben, Yellow eye and NY-69-6207-2 have some tolerance under field conditions (Anderson et al., 1974; Coyne et al., 1977b; Sherf and Macnab, 1986). Cvs. Ex Rico-23, Crestwood and Centralia were found resistant to white mould by Tu 1985 and 1989b. Schwartz et al. (1987) reported dry bean cvs. A-51, 83 VEF MXA222 and P.I.-169787 as tolerant during *in vitro* screening of dry bean calli. Hartman et al. (1987) found calli from lines P.I.-189567 and P.I.-226865 as tolerant to oxalic acid medium (putative toxin). The French bean cvs. Biola, Wyborowa, Wiejska and Saxobel and the lines IGE-1179 and IGE-2179 are resistant to *S. sclerotiorum* (Pieta, 1990). Middleton et al. (1995) confirmed the relative resistance of accessions Rico-23, Ex Rico-23, NY-5223 and NY-5268. New sources of resistance are in accessions PER-22, Flair, Pearly Wonji, Shimi, Uribe, Redondo and P.I.-263958 of *P. vulgaris* during three years of seedling screening under artificial inoculation tests in growth cabinets. Saindon et al. (1996) registered navy bean cv. AC skipper as resistant to *S. sclerotiorum*. Myers et al. (1997) registered cvs.

VI-911 and VI-137 of black bean and Navy bean, respectively as resistant to *S. sclerotiorum*. The accessions, Dunav 1, Padej 1, Isobella, A195, NAB 19, IIPR-7585 and SIN 11 are the most suitable donors for white mold resistance (Kiryakov et al., 2002).

18.5.2 Lettuce

Cvs. like Gallega dhiver, Blonde a bord rouge, Great Lakes 54 and Tetue de Nimes have shown resistance. Additional resistance is available in several wild *Lactuca* species (Sherf and Macnab, 1986). The most resistant species to *Sclerotinia* are *Lactuca* spp. *L. dentata*, *L. perennis* and *L. serrma* obtained from Poland, Japan, France and Belgium respectively (Abawi et al., 1980). Hawthorne (1974) reported cos varieties with an upright growth habit are known to be resistant.

18.5.3 Cauliflower

Under Solan (H.P.) conditions, Dohroo (1988) reported cv. Janavon as resistant and Super Snowball, Sel-12, EC-173803 and EC-173807 as moderately resistant to stalk rot disease under artificial inoculation condition at curd initiation stage. Cvs. Early Winter, Adam's White head and EC-162587 are reported as highly resistant and lines RSK-1301 and MKS-l as moderately resistant to *S. sclerotiorum* (Sharma et al., 1995). Singh et al. (1995a) reported cvs. Janavon, EC-103576, EWAWH and EC-177283 as resistant.

18.5.4 Soybean

The cvs. Corsoy, Hodgson and Hodgson-78 are less susceptible to stem rot (Grau et al., 1982). Amongst the cvs. tested more than once by Boland and Hall (1987) and Nelson et al. (1991b), Maple Arrow, Maple Ridge, Ace, Maple Presto, Portage and McCall are most resistant to *S. sclerotiorum* under field conditions. Lockwood and Kao (1987) found cvs. Ozzie, Hodgson-78, Pella, Corsoy-79, Hardin and Corsoy as tolerant to white mould disease. Podkina et al. (1988) reported cvs. Plamya and Grant as resistant. Jiao et al. (1994) observed cvs. Dong Nong-37, 39 and 30 as most resistant to *Sclerotinia* under China conditions. Chaves et al. (1996a) found cv. Abyara as most resistant to *Sclerotinia*. The perennial *Glycine tomentella* has potential untapped sources for improving disease resistance in soybean (Hartman et al., 2000). AXN-1-55 is mid group II maturity germplasm line released for use as a parent due to its high level of resistance to *Sclerotinia* stem rot (Diers et al., 2006) at Illinois and Michigan. Maturity group I to III plant introductions, 153, 282, 189,

931, 196, 157, 398, 637, 417, 201, 423, 818, 561 and 331 of soybean have high level of resistance to stem rot in the USA (Hoffman et al., 2002).

Soybean lines/cvs. Mandaries (Ottawa), Maple presto, PI 248509B, PI 384942, PI 423893, PI 503336, PI 504497, PI 507327, PI 578496, PI 592949, PI 592953 and PI 594286 are the most resistant to *Sclerotinia* under Urbana, USA conditions (Manandhar et al., 1999). Cvs. Corsoy and S19-90 are consistently resistant at Ames, USA (Wegulo et al., 1998) and Korea Republic (Kim et al., 2000). The soybean genotypes NKS 1990, Asgrow, A2506, Colfax and Corsoy 79 have greatest resistance to sclerotinia stem rot in Korea Republic (Kim et al., 1999).

18.5.5 Safflower

Cv. AC Sunset has been registered as resistant to *S. sclerotiorum* head rot from Manitoba and Saskatchwan, Canada. Two restorer (RHA 439 and RHA 440) and maintainer (HA 441) germplasm lines have been registered as *Sclerotinia* head rot tolerant from North Dakota (Miller and Gulya, 2006).

18.5.6 Linseed

Cvs. Antares and Norlin show a high level of resistance against *S. sclerotiorum* in UK (Pope and Sweet, 1991).

18.5.7 Peas

Germplasm lines/cvs. PI-155109, PI-166188, PI-189171, PI-261622, PI262189, PI-263027, PI-272191, PI-272205, PI-272209, ID-89-1, ID-2, Dark Skin Perfection, Perfection-132 and Wisconsin Perfection are resistant to white mould disease (Blanchette and Auld, 1978). Sharma and Kapoor (1999b) identified genotypes Dark skin, Perfection, DPP 19, DPP 54, DPP 58, DPP 71, DPH 9, DPH 86, HPPC 95, P 3471, P 3496, P 3549, P 3611, P 3641 and P 3673 as resistant to *Sclerotinia* rot of pea.

18.5.8 Egg Plants

Breeding lines V-1198, V-1200, V-1687 and V-1740 have moderate resistance and line V-1755 is highly resistant to *Sclerotinia* (Kapoor et al., 1990b).

18.5.9 Alfalfa

Cvs. Nccrzd and NCMP-2 are tolerant to *Sclerotinia* crown and stem rot (Welty and Busbice, 1978). Locally adapted varieties of clovers and alfalfa are less damaged by *Sclerotinia* stem and crown rot (SCSR) than are introduced varieties (Djikstra, 1964; Valleau et al., 1933; Welty and Busbice, 1978). Pratt and Rowe (1995, 1998) registered MSR alfalfa cv. as resistant to *S. sclerotiorum* from Mississippi whereas Florida-5472 as least susceptible to *S. sclerotiorum* and *S. trifoliorum*. In Japan, *Sclerotinia* crown rot and stem rot resistant strains viz., LR-43112-7, CRSY 541-1, CR 488-3, Everest, Derby, Lutece, Furez 507 and Delta have been identified (Kanbe et al., 2002). Alfalfa varieties WL 414, WL 325, WK 323, CS 40A and CS 40R are resistant to *S. trifoliorum* in China (Yuan–Qing Hua et al., 2003).

18.5.10 Clover

Resistance to *Sclerotinia* is reported in tetrapoloid red clover (*Trifolium pratense* L.) and in some diploid varieties (Arseniuk, 1989; Dixon, 1975; Djikstra, 1964; Dabkeviciene and Dabkevicus, 2005). Red clover cvs. Suminskii, Mestnyl, Vazhskii Mestnyl and Resident Otofte have multiple resistance to several pathogens including *S. trifoliorum* (Drozdova, 1988). The most resistant cvs. in the field are Kenster (4X), Marino (2X), P-35.5 (4X), P-28.3 (2X), P-28.5 (4X), Tepa (4X), Temera (4X), St-448 (4X) and St-448 (2X). Resistant tetraploids are derived from resistant diploids (Arseniuk, 1989). Raynal et al. (1991) found good resistance in Temara, Tetri Lessam and Vanessa (tetraploids) and Diper, Kuhn, Noc, Albatross and Jutin (diploids).

18.5.11 Peanut

Cvs. /lines, Chico, NC-3033, Florigiant-I7165 (North Carolina breeding line), PI-343392, PI-371521, VA-71-347 and Chinese peanut germplasm (Virginia breeding line) have been found to show tolerance or less susceptibility to the disease (Coffelt and Porter, 1980, 1982; Porter et al., 1975, 1992). Lines NC-3033, VGP-l and cv. Virginia 81 were found resistant by Coffelt et al. (1980, 1982). According to Cilliers and Wyk (1999) in South Africa, Cvs. Cateto and Kwarts are most resistant to *Sclerotinia* stem blight. In Australia, germplasm lines Tx AG-4 and VA 93 B shows high level of resistance to both *S. minor* and *S. sclerotiorum* (Cruickshank et al., 2002). In USA, the advance breeding lines N92056C, Cvs Tamrun 98 (TX901417) and Perry (N98112C) have moderate to high levels of resistance to *S. minor* with higher yields (Akem et al., 1992; Lemay et al., 2002).

18.5.12 Sunflower

Interspecific amphidiploids using wild perennials (*H. maximiliani*, *H. nuttali*, *H. hirsutus*, *H. divaricates*, *H. gresseserratus*, *H. strumosus*) provides resistant lines. Wild type sunflower (*H. maximilliani*) has been identified as completely resistant to *S. sclerotiorum* (Henn et al., 1997). Degener et al. (1999) observed *H. tuberosus* and *H. argophyllus* as resistant source. In Slovakia, Ziman et al. (1998) identified sunflower hybrid Sunstar 277 as most resistant to *Sclerotinia* wilt. Miller and Gulya (1999) identified *Sclerotinia* tolerant sunflower germplasm lines HA 390, RHA 391, RHA 392, RHA 408, RHA 409, HA 410, HA 411 and HA 412 from USA. The inbred line 28r (Coming from *H. argophyllus*) is not only most tolerant against *Sclerotinia* basal stem and white head rot infection, but also it gives the best performance in oxalate and culture filtrate tests highlighting a specific resistance to oxalate (Baldini et al., 2001). Hybrids, Pioneer 6480 and Pioneer 6479 are least susceptible to *Sclerotinia* wilt (Mosa et al., 2000b). Germplasm screening indicated HA 61, Lovaszpatonia, GIR 104, Yugykorosi, Hybrids CM 526X, HA 61-1 and Sunstar 277 as most resistant while CM 361, CM 953-8-3, P_{21}, MS X HA 61, CM 526, CM 497, Chernyanka, PK 104175, PI 377530 and PI 38057 are resistant to wilt as well as head rot (Baibakova, 1989; Pandey and Saharan, 2005).

18.5.13 Rapeseed–Mustard

In India, *Brassica* genotypes Cutlon, ZYR-6, PSM 169, PDM 169, Wester, PYM 7, Parland, Tobin, PCR 10, Candle, Wester, Cutlass and Torch (Shivpuri et al., 1997, 2001) and mustard genotypes PCR 10, RW 8410, RW 9401, Hyola 401, PBN 9501, PWR 9541, Kiran, RH 9401, RH 492, RW 8410, PAB 9511and RGN 8006 are resistant to stem rot (Pathak et al., 2002; Ghasolia and Shivpuri, 2005). In Japan, rape cvs. Koganenatane, Aburamasari and Kizakinonatane have low level of disease incidence (Tetsuka and Ishida, 2000). From Czech Republic, Vitasek (1994) reported rape lines, OKEG 8, 94, POH 285 and H 243/33 as most resistant to *S. sclerotiorum*. In Poland, winter rapeseed (*B. napus*) cultivars, BOH 2600, Bermuda, Capio and Mohllan are resistant to *Sclerotinia* (Starzycka et al., 2004). However, Jedryczka et al. (1996) identified cvs. Bar, BOH 1592, BOH 1693, MAH 1391 and MAH 1592 as resistant. According to Starzycka et al. (1998), cvs. MAH 1996, Valesca, Passat, Liero and BKH 894 show lowest number of infected plants. Strains PNG 2170, MA 1615-1, MZL 236, BK 2466/93, MA 1649-1 are most resistant (Starzycka et al., 2000). In China, the double low rape (*B. napus*) cvs. Zhongchuang 9 and Xiangyou 15 show a high resistance to stem rot (*S. sclerotiorum*). Upon inoculation, the phenolic contents and chitinases activity in Xiangyou increases rapidly and are maintained for long time (Guan-ChunYun et al., 2003; Wang-Han Zhong et al., 2004).

18.5.14 Sweet Potato

The USA bred cv. Beauregard is significantly resistant to *Sclerotinia* rot (Lewthwaite and Wright, 2005).

18.5.15 Dolichos Bean

In India, under artificial inoculation conditions cvs. Arka, Vijay, 6009, 6022, 6802, 7011, 7020B, 7101, 7202, 8101 and Rajani are resistant to stem and pod rot caused by *S. sclerotiorum* (Prajapati et al., 2005).

18.5.16 Cucumber

In China, cvs. Ganfeng 2, Zhougnongz, C1 and A15 are resistant to *S. sclerotiorum* (Zhu-Jian Lan et al., 1999).

Chapter 19
Disease Management

Most diseases caused by *Sclerotinia* have not been managed consistently and economically. The explosive pathogenicity of *Sclerotinia* species under favourable conditions and the ability of their sclerotia to withstand adverse conditions allow them to be successful pathogens on many crops. In field crops management practices should be applied to increase attainable yield despite their association with high disease risk (Mila et al., 2003). Methods of disease management that have met with varying degrees of success are as follows.

19.1 Cultural Methods

19.1.1 Sanitation

Any method that reduces sclerotial inoculum can significantly contribute to an effective management programme. Sclerotia sometimes are harvested along with sunflower, pea, bean, rapeseed or other seeds. The use of certified seed will reduce chances of introduction of the pathogens into clean fields. Occurrence of commonly grown weeds in the arable crops such as soybean, sunflower, rapeseed and others may lead to increase in *Sclerotinia* inoculum density. Redistribution of inoculum in infested crop straw, cull seeds or other residue into fields should be avoided. With chemical soil treatments, reinfestation possibilities always exist and reduction of sclerotial numbers per se in a field may not lead to disease control. Control of broad leaf weeds in all crops is useful, because many weeds are hosts of *S. sclerotiorum*. In addition, weeds can create a denser canopy which in turn favours disease development. Planting minimal populations with wide plant spacing within rows, combined with convenient row spacing, reduces the development of new infections to a minimum. Gilbert (1991) found that burning of stubble at fall is a highly effective control measure to destroy sclerotial inoculum.

Soil containing infected plant debris and sclerotial material can adhere to animals' feet, farmers' boots and to farm implements (Adams and Ayers, 1979), so the potential exists for inoculum to be transported to uninfected fields. Keep

G.S. Saharan, Naresh Mehta, *Sclerotinia Diseases of Crop Plants: Biology, Ecology and Disease Management*,

footwear and machinery clean are obvious ways to reduce this potential, but little can be done about animals. Runoff water from irrigation can also carry sclerotia between fields (Adams and Ayers, 1979; Schwartz and Steadman, 1978), so some way of controlling the direction of drainage may be advisable. The manure from cattle which are given diseased plant tissue for feed and bedding also provides a source of inoculum if it is spread on uninfected fields (Adams and Ayers, 1979). So care must be taken that only healthy plant tissue is used or that the manure is not spread on the field. The greatest potential for long distance dissemination of inoculum is through infected seeds. The separation of seeds from sclerotia is based on the specific gravity of crop seed and sclerotia of *S. sclerotiorum*. In a bath at 35–40°C, the air held by the sclerotia escapes and sink. This operation takes only 5–6 min and provides 95–98 per cent clean seed or the seeds may be stirred in the warm water for 15 min with a wooden stirrer and the floating seeds are removed and dried (Lukashevich, 1961; Tripolka, 1977; Milenko, 1964).

19.1.2 Tillage Operations

Tillage operations have both positive and negative effects in reducing the soil population of sclerotia. It is reported that shallow harrowing retains infested residue on or near the soil surface and accelerates the reduction of the inoculum potential of the pathogen (Abawi and Grogan, 1975). Deep ploughing as opposed to surface cultivation can reduce the number of sclerotia present near the soil surface and hence reduce the number of sclerotia that can germinate (Abawi and Grogan, 1975; Williams and Stelfox, 1980b), but since sclerotia remain viable even at depths of 17.5 cm (Abawi and Grogan, 1975), deep tillage in subsequent years can bring these sclerotia back to the soil surface and thereby increase the level of inoculum (Partyka and Mai, 1962). Deep ploughing has been recommended for the control of white mould of bean, but ploughing to a depth of 25 cm does not affect disease severity in Nebraska and thus may be a valid general recommendation (Steadman, 1983). Furthermore, infected plant debris and cull seeds that are worked into the soil stimulate sclerotium formation again causing an increase in sclerotium numbers (Cook et al., 1975; Kruger, 1975b). Mouldboard ploughing plus mulch tillage (MP + MT) plots of soybean has the lowest disease incidence of *Sclerotinia* stem rot along with higher yield (Mueller et al., 2002). Deep ploughing is not successful disease management strategy for lettuce drop (Subbarao et al., 1996). The suppression of lettuce drop under subsurface drip irrigation is attributed to differential moisture and temperature effects rather than to changes in the soil microflora or their inhibitory effects on *S. minor* (Bell et al., 1998). Crop rotation and no tillage of soybean is the most useful combination of treatments that reduces the primary inoculum (apothecia) of *Sclerotinia* in infected fields (Gracia et al., 2002). However, according to Wu and Subbarao (2003), subsurface drip irrigation and associated minimum tillage makes it a valuable cultural practice for lettuce drop management.

19.1.3 Mulching of the Soil

In India *Sclerotinia* stalk rot of cauliflower reduces significantly by mulching the soil with pine needles and sunflower inflorescence residues (Singh, 1987). *Sclerotinia* disease of greenhouse grown eggplants and cucumber can be controlled by the use of a light filter that inhibits apothecial development. Covering the plants with UV absorbing vinyl film (lower limit of transmission 390 nm) reduces the total number of apothecia (Honda and Yunoki, 1977, 1980). Disease also is reduced under UV-absorbing vinyl film. Even the use of black plastic mulch placed over the soil and under the lettuce leaves decreases *S. minor* infection (Hawthorne, 1974).

19.1.4 Host Nutrition

Infection of sunflower plants is dependent on nutrition during growth. Placement of phosphorus during growth especially in mixture with humus reduces infection two to three times and considerably increases the yield (Lukashevich, 1964a, b; Polyakov, 1973). Micro-nutrients and slaked lime are also reported to increase resistance of sunflower plants to the disease (Kochenkova and Polyakov, 1971). Application of low rates of nitrogen such as 6 kg a.i./ha is associated with significant reduction of canopy size and density, less lodging and lower levels of *Sclerotinia* rot of carrot compared to 60 kg a.i. ha^{-1} with negligible effect on yield (Couper, 2001). The combination 120 kg N/ha and 40 kg S/ha significantly reduces *Sclerotinia* rot of mustard (Gupta et al., 2004a).

19.1.5 Crop Rotation

Crop rotation is a disease control recommendation and often has been advocated for control of *Sclerotinia* diseases. The crop history of a field is a major factor in determining the level of inoculum in the soil. Due to long term sclerotium survival, soil populations of sclerotia remain relatively stable for at-least three years of a non-host crop following a host crop (Dueck, 1977; Morrall and Dueck, 1982; Schwartz and Steadman, 1978; Williams and Stelfox, 1980b). In general, lower mean numbers of apothecia and (or) clumps of apothecia are observed in plots planted with maize or winter wheat than with soybean (Gracia-Garza et al., 2002). However, the consecutive years of a host crop such as rapeseed increases sclerotium numbers when compared to only one year of the host crop (Williams and Stelfox, 1980b). Thus, crop rotation may be useful in preventing an increase in the soil population of sclerotia, but it is ineffective in reducing the number of sclerotia already presents in the soil. It is believed that crop rotation may be more effective on *S. minor* that infects from sclerotia rather than from ascospores. Crop rotation with broccoli can be a practical lettuce drop management strategy (Hao et al., 2003).

It is reported that under Manitoba conditions in canola, at least five years should separate sunflower from the preceding susceptible host crop, suggesting five years crop rotation with non-host crops such as barley, wheat, beets and flax (Zimmer and Hoes, 1978). Similar reports suggesting three to four years crop rotation with non-host crop have been made from Austria (Krexner, 1969). The number of sclerotia in a field can increase even in the absence of a host crop if there are host weeds present in the non-host crops grown in that field during the crop rotation period (Kruger, 1980; Morrall and Dueck, 1982). A good weed control programme can, however, eliminate this source of inoculum. In India, minimum white rot of mustard is recorded in pearl millet–wheat-pearl millet-raya rotation (Sharma et al., 2001). In USA, alternate crops of broccoli and lettuce provides greater reduction in the soil borne sclerotia than consecutive crops of broccoli and lettuce (Subbarao et al., 1998)

In India, the cauliflower-rice-cauliflower or cauliflower-maize-cauliflower rotations are good to reduce disease severity (Singh, 1987). However, Gupta et al. (1987) recorded maximum reduction in stalk rot of cauliflower under Solan (India) conditions when rotation with rice is compared with rotation with maize, tomato and fallow. The rice rotation also gives increased yield.

19.1.6 Date of Planting

The incidence and severity of *Sclerotinia* stem rot and wilt of pea grain is significantly less in November sown crop than on other dates. Due to maximum germination, growth and profuse flowering and fruiting, the yields from such crops are higher when compared with those obtained from crops sown at other periods (Singh and Singh, 1984a). Early sowing of pea by 21 October under Indian conditions (Palampur, H.P.) results in high disease incidence. Late sown crop after November results in no disease development. Early sowing leads to profuse negative growth which forms a canopy providing microclimate conditions and senescent leaf and petal tissue suitable for infection (Singh and Singh, 1995). In India, January sown sunflower crop gets less disease in comparison to the crop sown in October-November because of favourable temperature during susceptible growth stage of the crop (Kolte and Tewari, 1977; Singh and Tripathi, 1995). In India, mustard crop sown between fourth week of October to first week of November shows minimum white rot severity (Gupta et al., 2004b; Ghasolia et al., 2004b; Sharma et al., 2001). Stem rot of berseem is effectively controlled by sowing the crop in November under Punjab, Indian conditions (Singh and Singh, 1995).

19.1.7 Moisture Regulation

Flooding a field continuously for 23–45 days or cycles of alternate flooding and drying led to destruction of sclerotia of *S. sclerotiorum* and reduces disease in Florida and India (Moore, 1949; Sherf and Macnab, 1986; Singh and Tripathi, 1996a). Liu and Sun (1984) and Moore (1949) while working on lettuce root rot

observed lysis of sclerotia in a continuously flooded field. However, this technique has limited usefulness in most non-irrigated areas. Smith (1972) found that sclerotia of *Sclerotinia* spp., when dried for short periods and remoistened in soil, leaks nutrients are rapidly colonized by microorganisms and decay in two to three weeks. Drying of *S. minor* sclerotia stimulates mycelial germination. For irrigated crops such as lettuce, there is usually less drop if beds are made higher to provide for rapid drainage of water. Reduction in the number of irrigations, especially those at the end of the season, can reduce disease in the absence of rainfall but final irrigation should not be eliminated unless disease potential is great. In a three year study conducted in Nebraska on irrigation frequency, it has been shown that both apothecium production and disease severity are reduced by less frequent irrigation of Great Northern bean cultivar (Blad et al., 1978; Schwartz and Steadman, 1978). Yield increase at the lower water rates is correlated with lower disease severity. Elimination or reuse of surviving irrigation runoff water can reduce the chances of spreading sclerotia, mycelia or ascospores from one field to another.

Irrigation can have a major impact on soil borne pathogens because of its influence on soil moisture (Rotem and Palti, 1969). Tube well irrigation water with low EC, Ca^{++}, Mg^{++} and $C1^{-}$ significantly reduces white rot of mustard (Sharma, 2004). A comparative study of furrow and subsurface drip irrigation on disease and yield of lettuce revealed a significant reduction in the incidence of lettuce drop under subsurface drip irrigation. In addition, yield of lettuce is significantly increased under subsurface irrigation (Subbarao et al., 1994, 1997). Significantly more *S. minor* sclerotia are added to the soil after each lettuce crop under furrow irrigation as compared with subsurface drip irrigation. Populations of sclerotia increase about fivefold after three years of conventional tillage under furrow irrigation. Spatial patterns of sclerotia are less aggregated in these plots than in drip irrigated plots. With subsurface drip irrigation, the distribution patterns and the numbers of sclerotia in plots are changed little by minimum tillage practices (Subbarao, 1996). Subsurface drip irrigation and associated mandatory minimum tillage practices significantly reduces the incidence of lettuce drop compared with furrow irrigation and conventional tillage. The suppression of lettuce drop under subsurface drip irrigation compared with furrow irrigation is attributed to differential moisture and temperature effects rather than to changes in the soil micro flora or their inhibitory effects on *S. minor* (Bell et al., 1998). According to Matheron and Porchas (2005), maintaining flooding event for two to three weeks in fields with a history of lettuce drop caused by *S. minor* and *S. sclerotiorum* can significantly reduce the population of viable sclerotia. Flooding offers effective control of *Sclerotinia* in pea seed crop in India (Sharma and Gill, 1996).

19.1.8 Host Row Orientation

Differences in incidence and severity of forage crown rot caused by *S. trifoliorum* on north and south facing slopes has been recorded by Bennett and Elliot (1972). Similarly, Haas and Bolwyn (1974) indicate effect of row orientation on bean white mould severity in Canada.

19.1.9 Soil Solarization

Solarization reduces the population of sclerotia of *S. sclerotiorum* in soil and reduces the ability of the surviving sclerotia to form apothecia. The greatest reductions occur in the top 5 cm layer of soil but significant effects are seen at 10 and 15 cm depths. These reductions are mainly due to microbial colonization and degradation of sclerotia weakened by the sub-lethal temperatures produced by solarization. A beneficial side effect is significant reduction in the population of weeds in solarized plots (Phillips, 1990). Solarization for 30 and 15 days affects recovery and viability of sclerotia in relation to depth of burial in the soil. Recovery of sclerotia after 15 days of solarization at a depth of 5, 15 and 30 cm is 32/60, 46/60, 48/60, respectively and viability of recovered sclerotia is 29, 45 and 52 respectively (Cartia et al., 1994).

During soil solarization, *S. sclerotiorum* sclerotia are completely killed at 45°C temperature after 3–4 h and at 35–40°C after 10–14 h. High temperature increases the exudate from sclerotia due to the high bacterial population on the cell surface. Soil mixed with 1, 0.5 or 0.1 per cent calcium cyanamide reduces sclerotial survival. Disease incidence is greatly decreased by treating with Ronilan (Vinclozolin), soil solarization + Vinclozolin or soil solarization + calcium cyanamide (Chen and Wu, 1990). Solarization of soil during May and June in India reduces the population (89.2–98.3 per cent) of *S. sclerotiorum* in soil and ability of surviving sclerotia to form apothecia (90.0–98.6 per cent). The greatest reduction (100 per cent) occurs in the top 7.5 cm layer of soil after eight weeks but significant effects are seen at 15 and 30 cm soil depth after nine weeks (100 and 93.3 per cent) soil solarization (Sharma et al., 2005). Soil solarization is a common cultural practice in New Zealand to reduce viability of sclerotia of *S. sclerotiorum* (Swaminathan et al., 1999). Soil solarization reduces the incidence of lettuce drop by 82 and 67 per cent (Table 19.1.9.1) in the field (Gepp et al., 2001). Covering soil after the application of metham and clozonet with low density polyethylene films improve efficacy of fumigants and reduces duration of solarization (Gullino et al., 1998). According to Wu (1991) soil solarization with black polyethylene sheets is effective in reducing the number of apothecia of the pathogen. The temperature of the top soil in the solarized field rises by 10°C and by 5°C at a depth of 5 cm. Sclerotia loose their viability in wet soil kept at 45°C for four days.

19.1.10 Microclimate Modification

It is a very promising and effective way in which sclerotium germination and apothecium formation can be inhibited, hence it is an effective way to reduce the level of disease in a host crop. Since sclerotia require moisture to germinate and form apothecia, any way in which the area under a crop canopy can be kept relatively dry would be an asset in reducing both apothecium production and disease development. This may be a difficult task to accomplish in irrigated crops since irrigation creates the moist environment required under a crop canopy for disease development even when

Table 19.1.9.1 Effect of solarization on incidence of lettuce drop (*Sclerotinia* sp.) in the three experiments (Adapted from the publication of Gepp et al., 2001. With permission)

Field, 1998–1999		Field, 1999–2000		Greenhouse, 1999–2000	
Solaziration (days)	Diseased plants (%)	Solarization (days)	Diseased plants (%)	Solarization (days)	Diseased plants (%)
0	31.04a*	0	28.33 a	0	5.42 a
60	5.42 b	30	10.78 b	20	2.36 b
90	5.02 b	45	7.62 b	30	0.78c
				30 + org.[a]	0.93 c

*Means followed by different letters in the same experiment are significantly different ($P < 0.05$)
[a]With poultry litter added prior to solarization

the gross environment is unfavourable (Rotem and Palti, 1969). Reducing the amount and frequency of irrigation is effective in reducing disease development since it would allow the microenvironment under the crop canopy to dry out periodically and thus arrest disease development. Watering bean plants thoroughly until a continuous canopy forms, then reducing irrigation amount and frequency later in the season will result in less white mould and a stable yield (Steadman, 1979, 1983).

An association between plant canopy development and *Sclerotinia* disease incidence and severity has been observed in various crops. Susceptible lettuce cvs. produce a canopy that creates a favourable microclimate and also provides senescent leaf tissues for infection by *S. minor* (Hawthorne, 1974), although it can also attack from below ground (Marcum et al., 1977). Similarly, the effects of row spacing, growth habit and plant density on bean and potato canopy development, disease incidence and severity are reported in Nebraska (Coyne et al., 1974; Steadman et al., 1973) and New York (Partyka and Mai, 1962) respectively. A study comparing the microclimates of two dry edible bean canopy types in a semiarid region revealed that the vigorous viny cv. produces the most dense canopy and when irrigated heavily is the coolest and wettest and produces the highest disease severity (Blad et al., 1978). Crop density is an important factor when considering microclimate modification (Rotem and Palti, 1969).

Canopy clipping reduces the quantity of apothecia in the crop by creating an unfavourable microclimate for the development of *S. sclerotiorum* without affecting the fresh foliar and root weight of carrot at harvest (Kora et al., 2005a). Removal of foliage reduces *Sclerotinia* blight in peanut and increases diseases control sprayed with fluazinam (9.2 kg a.i. ha^{-1}) in the fields with high disease pressure (Butzler et al., 1998). In China, removal of all leaves of rape under 1 m of the main stem increases yield by 6–15 per cent (Fu and Tang, 1994).

19.1.11 Host Growth Habit

Plant growth habit is not an important factor in reducing disease as microclimate conditions. Bean cvs. with indeterminate growth produce more blossoms and have

more colonized senescent tissues than those of determinate growth but this is not always correlated with disease severity. Instead, the canopy structure and crop density associated with the respective growth habits determine whether or not the microclimate created is favourable for infection and disease development (Schwartz et al., 1978). Upright indeterminate and open bush type also results in reduced production of apothecia as compared with that under dense compact bush or vine types (Schwartz et al., 1978). Wider row spacing reduces canopy density and white mould disease in beans (Steadman et al., 1973). In dry beans growing cvs. with a strong basal stem and narrow, upright growth controls white mould disease (Huang and Kemp, 1989). Cultivars or lines with combined physiological resistance and upright growth habit such as cv. AC Skipper and U 1906 are generally the least susceptible to white mould even under high disease pressure (Huang et al., 2003). The incidence of *Sclerotinia* stem rot is reduced significantly when blossoms are removed from plants before blossom drop. Flower blossoms are sown to be a paramount bridge between air borne ascospores of *Sclerotinia* and stem infection in the potato canopy (Atallah and Johnson, 2004).

19.1.12 Host Population and Spacing

Crop density is an important factor in determining disease incidence and severity. Planting fewer plants per row and increasing the row spacing should, therefore be an effective way to increase the rate of evaporation and thereby decrease the length of time where favourable conditions for disease development exist (Coyne et al., 1974; Steadman et al., 1973). When the bean cv. Aurora and cvs. of Great Northern type are grown at a within row spacing of 30.5 cm, both are equally susceptible to *S. sclerotiorum*. However, at a spacing of 45 cm, Aurora contracts less disease symptoms than does the Great Northern cvs. (Coyne et al., 1977b). Four peanut cvs. Tamspan 90, Southwest Runner, Okrun and Flavor Runner 458 at four plant spacing (6, 15, 30 and 60 cm) shows increase in disease incidence with increased spacing (Maas et al., 2006).

In soybean disease severity index is lower with high yield if plant population is reduced from 5,60,000 seeds/ha to 4,30,000 seeds/ha in 19 cm rows. Plant population is positively correlated with DSI ($r^2 = 0.33$; $P = 0.001$) and negatively correlated with yield $r^2 = 0.33$; ($P = 0.0140$). Reduction of soybean population is more important than increasing row spacing to manage *Sclerotinia* stem rot in an irrigated system (Lee et al., 2005).

In sunflower, wider plant spacing is beneficial in reduction of the disease incidence. Yield reductions due to wilt are lower at densities 27,500 to 55,000 plants/ha than at densities of 82,000 plants/ha and higher (Hoes and Huang, 1976). A lot of free air is circulated in the widely spaced plants which in turn hastens the drying of soil and apothecia. This helps in reduction of development of head rots. Similarly, in the widely spaced plants contact between roots of healthy and adjoining diseased plants is reduced resulting in less incidence of wilt (Huang and Hoes, 1980; Young

and Morris, 1927). The use of plant spacing of 36 cm or greater and plant populations of 26–49 × 10^3 plants/ha can minimize yield loss due to *Sclerotinia* wilt (Hoes and Huang, 1985).

19.1.13 Burning of Stubbles

Burning of stubbles does not appear to be an effective method of control of *Sclerotinia* stem rot in the field (Hind-Lanoiselet et al., 2005).

19.2 Seed Treatment

Seed treatment is advocated to control *Sclerotinia* from infested rapeseed (Alabouvette and Louvet, 1973). However, benefits of seed treatment are questionable because of the low incidence of disease resulting from sunflower (Alabouvette and Louvet, 1973) and bean seed (Steadman, 1975) inoculum. In sunflower, seed treatments with fungicides such as carbendazim and thiophanate-methyl (Vernuseu and Iliescu, 1980), Benomyl (Auger and Nome, 1971; Sharifi-Tehrani, 1974), Thiram (Lukashevich, 1964a; Yakutkin, 1991a), Mancozeb (Sharifi-Tehrani, 1974), Zineb (Ale-Agha, 1974) and organomercurials (Lukashevich, 1964a) have been reported to be effective for control of the disease. Treatment of seed with Cd-acetate or Cd-chloride has also been found effective in elimination of infection (Crisan, 1964). The possibility of control of the disease through seed treatment with Quinolate-V-4x (300 g/1,000 g seed), Busan EC 30 (300 ml/1,000 g seed) and Lekind 15 S (300 g/1,000 g seed) has also been indicated (Acimovic, 1979).

In pea, seed treatment with molybdenum and boron and a combined application of molybdenum with Fundezol (Benomyl) gives good control of *S. sclerotiorum* with increase in yield (Kuleshova, 1990). Seed treatment with carbendazim @ 2.5 g/kg controls *S. sclerotiorum* of pea (Sugha, 1999). However, according to Handoro et al. (2002), seed treatment of pea with *T. harzianum* is superior to soil application in controlling the *S. sclerotiorum* infection more than 71 per cent.

In soybean, under field conditions fungicides, Thiram, Fludioxanil and Captan + PCNB + Thiabendazole reduce sclerotia formation from infected seeds by more than 98 per cent (Mueller et al., 1999).

Warm water treatment of seed at 50–60°C for 10–15 min is reported to be effective for disease control (Krexner, 1969). Acidenzolar–s-methyl (BTH) and methyl jasmonate (MeJA) applied to melon seeds may activate on seedling diverse metabolic pathway leading to the enhancement of resistance against *S. sclerotiorum* (Buzi et al., 2004).

Mustard seeds dressed with *Trichoderma viride*, *T. harzianum* and *Gliocladium virens* plus carbendazim 50 WP or mancozeb significantly increases per cent germination, plant height, dry plant weight and test weight of seeds (Fig. 19.2.1) has

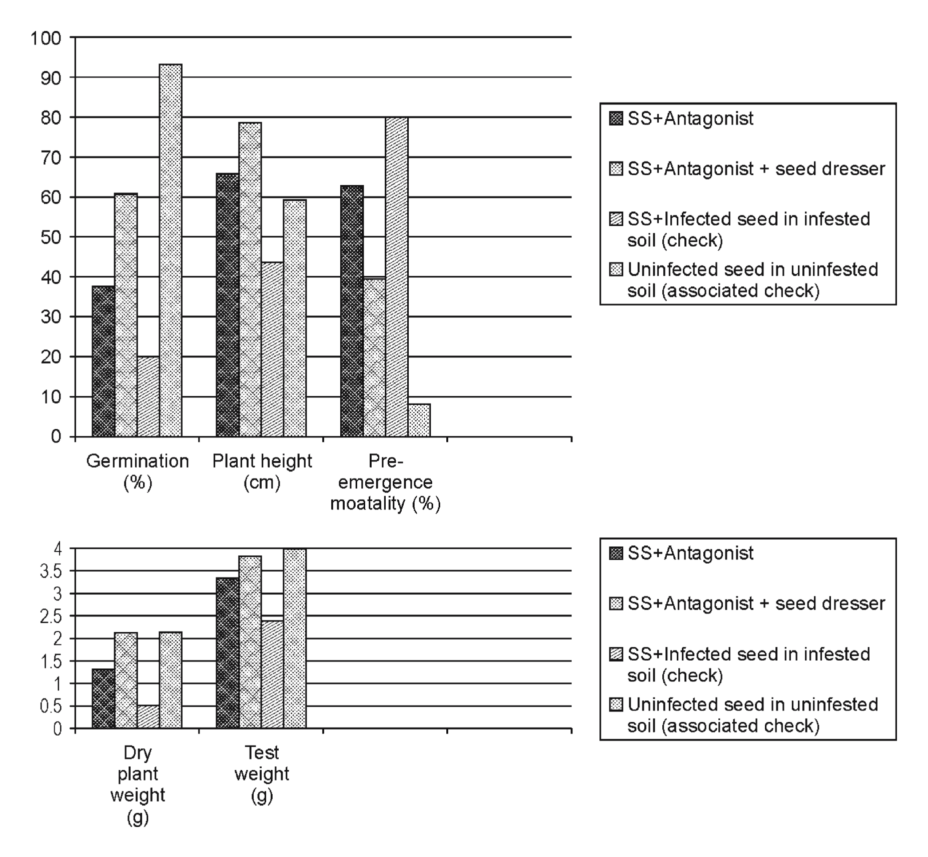


Fig. 19.2.1 Effect of antagonistic fungi and seed dressing fungicides on the germination and plant growth parameters in mustard (Adapted from the publication of Pathak et al., 2001. With permission)

been reported by Pathak et al. (2001). Maximum reduction in white mold (*S. sclerotiorum*) of French bean is observed by Nath et al. (2004) when seeds are sown after treatment with talc based formulation of *T. harzianum* + carboxyl methyl cellulose (CMC) + sub-lethal dose of carbendazim (0.05 and 0.10 per cent).

19.3 Soil Treatment

Methyl bromide or formaldehyde has been used as effective pre-plant treatments for destroying sclerotia in the soil (Alabouvette and Louvet, 1973). Calcium cyanamide (500 kg ha^{-1}) has been widely reported to prevent sclerotial germination and subsequent ascospore production (Kruger, 1973). Brooks et al. (1945) found that application of cyanamide at 800–2,000 lb/acre gives good control of white mould

of beans in muck soils of Florida. In marl soils infection is reduced with 500–700 lb/acre of cyanamide (Moore et al., 1949). Gabrielson et al. (1973) reported significant reduction of *S. sclerotiorum* infection in cabbage seed plants with a single ground application of 1,123 kg/ha cyanamide. The treatment provides control even when the plots are located within 30 m of untreated infested areas. In Germany, experiments designed to study the efficacy of soil application of PCNB for control of *Sclerotinia* on rape were confounded by aerial inoculum from outside the treated areas. Even in the absence of aerial spore showers, furrow irrigated disinfested fields can be reinfested by sclerotia or ascospores in reused irrigation runoff water. In dry edible beans, application of PCNB results in a reduction of apothecial inoculum produced within the same field but there is no concomitant reduction in disease or yield increase. Use of soil fumigants not only has been ineffective in controlling diseases caused by *S. sclerotiorum,* but Partyka and Mai (1962) reported that fumigation with dichloropropene-containing compounds actually increases the incidence of lettuce drop. Where lettuce drop is caused by *S. minor*, methyl bromide, as a result of its destruction of inoculum, can reduce disease. Hartill and Campbell (1973) controlled *Sclerotinia* in tobacco seedbeds by fumigation with either MB or DMTT and by drenches of either Benomyl or thiophanate-methyl. According to Ben-Yephet (1988), methamsodium, MES (35 ml/m^2), methyl bromide (MB-50 g/m^2) and soil solarization kills sclerotia in the top 10 cm soil and reduces apothecial production. Certain chemicals such as quintozene, fentin acetate and calcium cyanamide have been found effective to inhibit apothecial development of *S. sclerotiorum*. The efficacy of calcium cyanamide in controlling the disease in rapeseed by 40–90 per cent has been confirmed under field conditions in Germany (Hara and Yanagita, 1967; Kruger, 1973, 1974, 1980). Control of disease through the use of procymidone is also reported (Brun et al., 1983). Soil incorporation of Dazomat completely inhibits apothecial production and no sclerotia are recovered 20 weeks after burying (Table 19.3.1). Calcium cyanamide and Quintozene significantly reduces number of apothecia and sclerotia recovery (McQuilken, 2001). However, the combined soil incorporation of Quintozene and foliar sprays of Iprodione significantly improves disease (Table 19.3.2) control compared to either treatment applied alone (McQuilken, 2001). Ninety five per cent control of *S. sclerotiorum* has been obtained in Germany by applying 40 g Dazomet/m^2. Dazomet is very effective against sclerotia of *S. sclerotiorum* at 5–20°C. Dazomet prevents regeneration of the black rind on the sclerotia (Jones, 1974b). Developing sclerotia of the fungus exude a clear liquid which contains the enzyme o-diphenol oxidase. The activity of this enzyme, which is also present in the sclerotial tissue is inhibited by Dazomet, sodium azide and DIECA. These inhibitors can be prevented in the presence of sufficient quantities of Cu^{2+}. The activity of mushroom o-diphenol oxidase is affected by Dazomet and Cu^{2+} in a similar manner (Vaughan and Jones, 1979).

Application of Nitrolim (12 mg/pot) which contains 57 per cent calcium cyanamide and Benlate and Benzotriazole (5 mg/pot) to the soil surface completely inhibits germination of sclerotia of *S. sclerotiorum*, whether buried or placed on the soil surface, over a period of at least 20 weeks at 20°C (Jones and Gray, 1973). In general, Vinclozolin applied at the rate of 0.5 kg a.i./ha in the soil is the best with

Table 19.3.1 Effect of different soil incorporations on apothecial production and percentage recovery of sclerotia of *S. sclerotiorum* (Adapted from the publication of McQuilken, 2001.)

Soil incorporation	Sum of apothecia	% Sclerotia recovered
Control (nil)	30 ± 1.8	93 ± 1.8
Calcium cyanamide	8 ± 2.2	61 ± 5.3
Dazomet	0	0
Quintozene	5 ± 1.5	64 ± 3.7

(Mean ± SE, n = 10)

Table 19.3.2 Effect of single and combined applications of soil and foliar applied fungicides on *S. sclerotiorum* diseased lettuce plants (Adapted from the publication of McQuilken, 2001.)

Treatment	% Diseased
Control (nil)	41 ± 3.1
Quintozene (soil incorporation)	20 ± 3.6
Iprodione (2-spray as foliar application)	23 ± 1.5
Quintozene + Iprodione	12 ± 1.3

(Mean ± SE, n = 4)

an efficacy of 100 per cent in inhibiting stipes and apothecia of *S. sclerotiorum* sclerotia (Costa et al., 2004).

Naar and Kecskes (1997) recommended combined application of Vinclozolin and the antagonist *T. viride* for better disease control caused by *S. minor*. To control damping off of bean plants, liquid swine manure, *Bacillus subtilis* (10^8 cfu/ml) and Iprodione (0.1 per cent a.i.) gives best control (Viana et al., 2000).

In lettuce, soil treatment with Iprodione or Vinclozolin (100 g/m^3) should be followed after planting. Depending on soil infestation two to three leaf sprays with Iprodione or Vinclozolin or Procymidone at 1.5 kg/ha before the 18 leaf stage should be provided (Davet and Martin, 1980). Grill (1979) in France recommended soil treatment with quintozene at 1.5 kg a.i./ha before planting against *Sclerotinia* spp. Five hundred kilograms per hectare calcium cyanamide gives 90 per cent reduction in incidence of *S. sclerotiorum* in lettuce with a concomitant increase in quality. Long-term use of calcium cyanamide increases soil fertility (Klasse, 1993). Thirty and 40 g/m^2 of methyl bromide (MB) applied by using virtually impermeable films to cover soil permits to achieve a very good control of *S. sclerotiorum* on lettuce in Italy (Gullino et al., 1996). The use of soil surface applied $Ca(OH)_2$ with fungicides, rotation and drip irrigation offers an opportunity for enhanced and sustainable control of lettuce drop (Wilson et al., 2005).

For the control of *Sclerotinia* disease of tomato in the greenhouse, steaming moist soil for 1 h at 131°F or treating with chloropicrin, metham sodium, di-trapex, or dazomate kills the sclerotia but these procedures are not economically feasible outdoors (Sherf and Macnab, 1986). In most crops, one application of a fungicide such as benomyl, DCNA or PCNB can be economical if disease reduction is satisfactory.

In peanut, metham has been found to control the disease through irrigation water (Krikun et al., 1980). Post-emergence application of dinitrophenol herbicides such as dinoseb and naptalam and dyanap (one part dinoseb + two parts naptalam) are found to provide effective control of the disease when the herbicides are applied as broadcast at 0.84 kg/ha (Porter and Rud, 1980).

Sclerotinia disease of greenhouse grown eggplant and cucumber can be controlled by the use of a light filter that inhibits apothecial development. Covering the plants with UV absorbing vinyl film (lower limit of transmission, 390 nm) reduces the total number of apothecia when compared with covering plants with common agricultural vinyl film with lower limit of transmission i.e., 300 nm (Honda and Yunoki, 1977, 1980). Disease is also reduced under UV-absorbing vinyl film. Even the use of black plastic mulch placed over the soil and under the lettuce leaves decreases *S. minor* infection (Hawthorne, 1974).

In sunflower, soil application of benomyl and calcium cyanamide at 0.25 kg/ha has been found to reduce the incidence of the disease (Auger and Nome, 1971; Lukashevich, 1964a).

The application of benomyl (0.5 kg a.i./ha), benomyl + mancozeb (0.5 + 1.6 kg a.i./ha), Iprodione (0.75 kg a.i./ha) and methyl thiophanate (1.5 kg a.i./ha) through irrigation water controls white mould of beans (Oliveira et al., 1995). Pre-sowing application of carbendazim @ 10 kg/ha controls *Sclerotinia* rot of pea (Sugha, 1999).

Soil application of pesticides can be used in conjunction with bioagents of *S. sclerotiorum* in an integrated approach to disease control without toxic effect on the biocontrol agent (Adams and Wong, 1991). In soil infested with *T. koningii*, the numbers of viable sclerotia are reduced by 100 per cent within 60 days (Dos Santos and Dhingra, 1982). Addition of alginate pellets containing mycelial fragments of *T. harzianum* strain Thz 1D1 to soil increases the proportion of sclerotial colonization under field conditions (Knudsen et al., 1991).

Surface application of urea to soil at 25–150 kg/ha is effective in controlling carpogenic germination of sclerotia. Ammonia released from decomposition of the urea appears to be the key toxic agent responsible for the inhibition of germination (Huang and Janzen, 1991).

19.4 Soil Amendment

Compost prepared from municipal sewage sludge is a valuable resource that can provide macro nutrients and minor plant nutrients and improve the tilth and productivity of agricultural soils. Composted organic matter also reduces diseases caused by several soil borne plant pathogens including *Sclerotinia*. Greenhouse studies have shown that percent infection of lettuce seedlings by *S. minor* is reduced consistently, 40–50 per cent by adding 10 per cent sewage sludge compost to the potting soil containing the pathogen (Lumsden et al., 1983). Amendment of soil with composted sewage sludge significantly reduces the incidence of lettuce drop caused

by *S. minor* in the field as tested by Lumsden et al. (1986) during a four year period in both spring and fall plantings (Fig. 19.4.1). Compost is added to the soil in the first two years and residual effects occur in the final two years. Suppression of disease is correlated with soil microbial activity, total nitrogen, phosphorus, magnesium, calcium and organic matter content of the soil. The suppression effect of compost on disease of lettuce caused by *S. minor* is complex and may be related to improved physical structure or modified nutrient content of the soil, resulting in increased soil microbial activity (Lumsden et al., 1986).

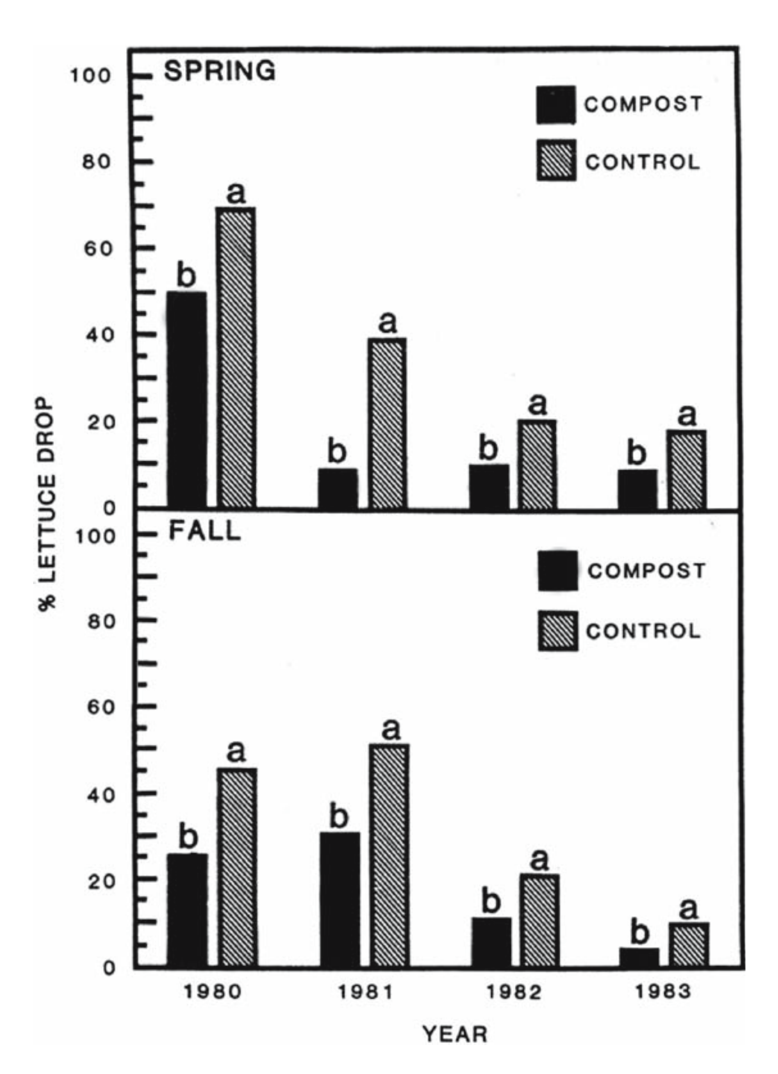


Fig. 19.4.1 Per cent lettuce drop caused by *Sclerotinia minor* in soil amended with composted sewage sludge or in nonamended soil in spring and fall plantings over a four years period (Adapted from the publication of Lumsden et al., 1986. With permission)

Among organic soil amendments tested, stable manure, fowl manure and lucerne hay are the best to reduce disease along with increase in yield of heads (Asirifi et al., 1994).

According to Huang et al. (1997a), an amendment of field soil with either a formulation of fermented agricultural wastes, CF-5 or allyl alcohol at 150–400 ppm suppresses apothecial production of *S. sclerotiorum* and enhances the colonization of sclerotia of the pathogen by *Trichoderma* spp. Organic soil amendments combined with biological control agents such as *C. minitans* and *T. virens* enhances the control of apothecia of *S. sclerotiorum* by killing of sclerotia through mycoparasitism (Huang et al., 2002). Singh and Tripathi (1996b) have suggested use of 2 per cent neemguard along with usual nitrogen application in the soil to manage *Sclerotinia* rot of sunflower. Incidence of stalk rot of cauliflower reduces by soil amendments of sunflower and rapeseed cakes and by removal of infected leaves at weekly intervals (Sharma and Sharma, 1986b). Singh et al. (1990) found that soil application of chopped leaves of *Aegle mormelos* 30 days before sowing is most effective in reducing chickpea stem rot by *S. sclerotiorum*. Soil amendment by S-H mixture and Perlka Red (calcium cyanamide) effectively controls *S. sclerotiorum* (Huang and Sun, 1991; Huang et al., 2006) of bean and canola at the rate 30 g/m^2 to 60 g/m^2 through reduction of carpogenic germination of sclerotia production of apothecia. Soil amendment with organic substances such as sweet clover straw (2.5 t/ha) or fish meal (0.8 or 2.5 t/ha) reduces the carpogenic germination of sclerotia of *S. sclerotiorum* and reduces the incidence and severity of white mould of bean (Huang et al., 2005a). Soil amendments with the Bougainvillea and Mehndi leaves reduces the pre and post emergence damping off, number of apothecia appearance, lesion length and disease intensity as compared to control. Similarly amendments with mustard cake, sesamum cake, cotton cake and jamun seed powder reduces the seedling mortality and these treatments were better than seed treatment with Bavistin (Hieu, 2007).

19.5 Herbicides in Disease Control

Although pesticides are applied to plants and soils to control plant parasites and weeds. These may also affect soil properties, microorganisms and hosts. These effects normally have little influence on plant growth and generally prove beneficial. It is important to ascertain the effect of each new pesticide on the chemical and biological properties of soils. Also important are factors influencing the persistence and decomposition of pesticides in the soil and the overall effects on soil microorganisms. Naito and Tani (1952) tested the effect of 2, 4-D on the mycelial growth, sporulation and sclerotial formation of various fungi including *S. sclerotiorum*. As a general rule, growth is inhibited between 0.32 per cent and 0.005 per cent (3,200 and 50 ppm). Apothecium formation is a critical stage in the life cycle of *S. sclerotiorum* because of the importance of ascospore inoculum in the initial infection of host plants. This stage is also the most sensitive to external influences. Divalent

metal ions, simple sugars, common salts, buffers and fungicides inhibit carpogenic germination generally at lower concentrations than are inhibitory to mycelial germination or growth (Hawthorne and Jarvis, 1973; Jones, 1973; McLean, 1958a; Steadman and Nickerson, 1975); carpogenic germination and the number of stipes produced in soil amended with Atrazine or Simazine, but apothecial disc development is inhibited (Radke and Grau, 1982, 1986). The specificity of inhibition by Atrazine or Simazine is in contrast to the effects of metribuzin and trifluralin, which increases production of both stipes and apothecia. These triazine compounds are effective herbicides that inhibit photosynthetic electron transport in plants (Ashton and Crafts, 1981). According to Casale and Hart (1986), mycelial growth of *S. sclerotiorum* on agar is inhibited by metribuzin and diuron at 50 μg/ml. The number of sclerotia that produce stipes is reduced by these compounds at 100 μg in soil (Figs. 19.5.1, 19.5.2). Abnormal apothecia are formed in soils amended with Atrazine (4–500 μg/g) or in solution of 2–50 μg M Atrazine. When normal immature apothecia grown in the absence of Atrazine are soaked in 50 μM Atrazine for 30 min, the hymenia abort and each give rise to new stipes. Huang and Blackshaw (1995) observed abnormal (apothecial) morphogenesis involved by Simazine and Atrazine at 25–50 per cent of the recommended field rates of 1,500 g/ha. Cerkauskas et al. (1986) tested 21 pre- and post emergent herbicides for their effects on linear growth of mycelium and production of sclerotia by *S. sclerotiorum*. None of the herbicides stimulate mycelial growth but most of them significantly retard growth. Sclerotial viability declines with EPTC at 20 μg a.i./ml (Tables 19.5.1–19.5.3) and trifluralin at 25 μg a.i./ml. 2-methyl-4, 6-dinitrophenol (DMOC) at 0.5 and 0.3 per cent (5,000 and 3,000 ppm) inhibits the formation of sporodochia of *S. laxa* on

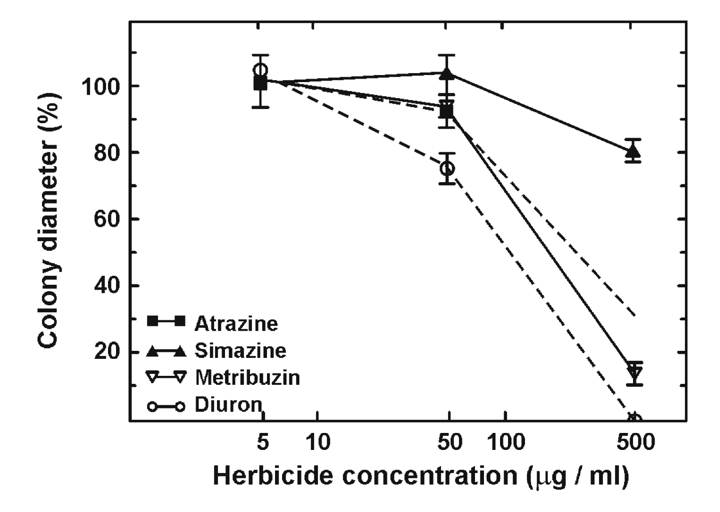


Fig. 19.5.1 Colony diameter of *Sclerotinia sclerotiorum* grown on herbicide amended water agar for three days as against percentage of unamended control (Adapted from the publication of Casale and Hart, 1986. With permission)

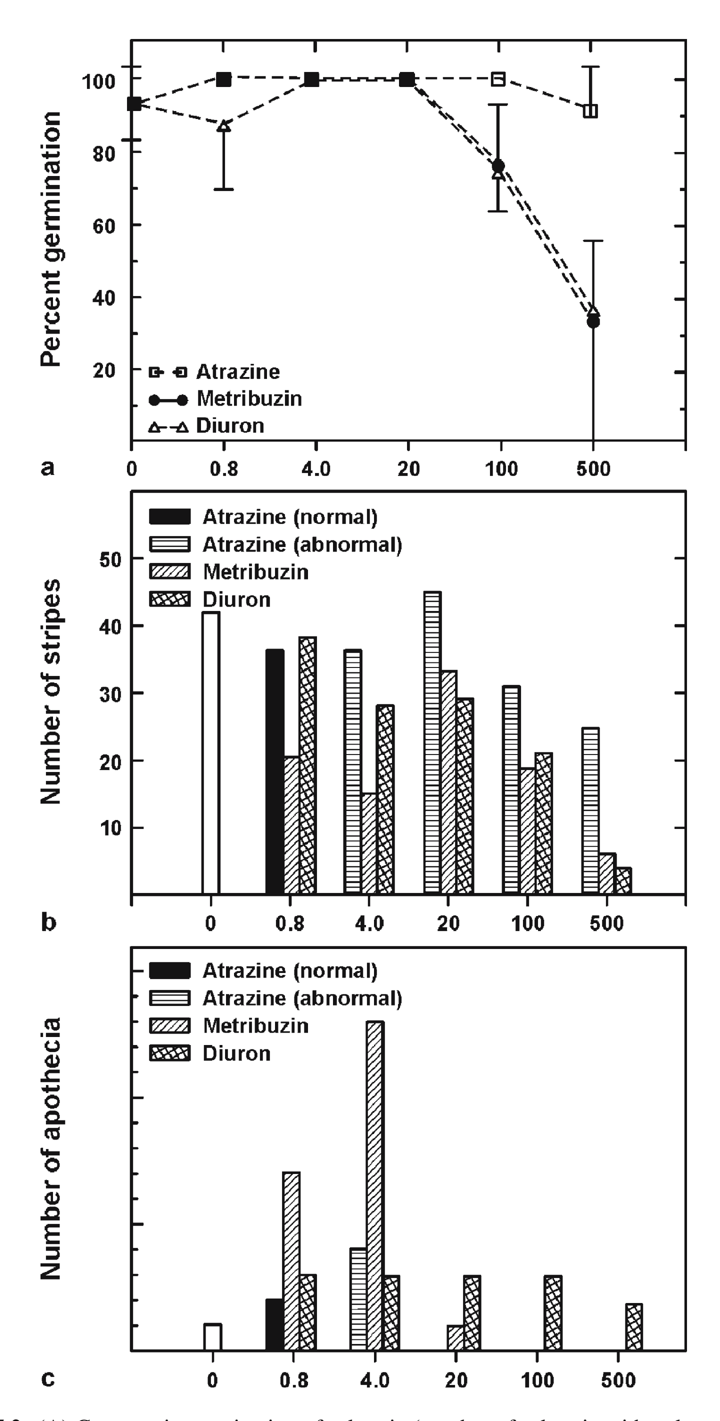


Fig. 19.5.2 (A) Carpogenic germination of sclerotia (number of sclerotia with at least one stipe per 20 sclerotia) of *S. sclerotiorum* incubated in herbicide amended soil for 27 days in the dark; (B) Stipes produced by 20 sclerotia incubated in herbicide amended soil for 27 days in the dark. Atrazine bars represent total number of stipes to that treatment; (C) Apothecia produced by 20 sclerotia incubated in herbicide amended soil for 28 days in the dark then for 18 days under fluorescent light (Adapted from the publication of Casale and Hart, 1986. With permission)

Table 19.5.1 Rate of mycelial growth of *Sclerotinia sclerotiorum* on potato-dextrose agar amended with various concentrations of pre or post emergence herbicides (Adapted from the publication of Cerkauskas et al., 1986. With permission)

	Growth rate (cm/48 h) per herbicide concentration (μg a.i./ml)									
Herbicide	0	1	2.5	5	10	25	50	100	500	1,000
2,4-D ester	12.6[a]	10.1	8.6	8.6	10.0	9.0	6.3	6.0	0.8	0
2,4-DB	11.2	5.2	4.7	4.4	3.9	1.7	1.4	0.9	0.6	0.6
Alachlor	11.2	5.6	6.9	8.8	6.6	5.2	2.9	2.2	1.0	0.7
Atrazine	11.6	5.9	5.8	5.6	5.0	5.3	5.0	3.3	1.7	1.3
Barban	7.5	5.6[b]*	3.3	2.6	1.6	0.7	0.7	0.1	0	0
Benazolin	12.2	4.0	7.5	10.7*	6.8	8.4*	4.7	3.9	0.8	0.8
Bromoxynil	12.2	7.6	4.6	6.7	5.3	1.9	1.4	0.9	0	0
Cyanazine	12.6	4.2	11.4*	11.7*	11.3*	9.5	9.9	10.2	2.2	4.1
Dalapon	12.6	8.4	10.1	11.5*	11.1	10.5	10.2	10.8	8.9	1.2
Diallate	12.6	10.7*	8.9	10.6	8.8	7.8	5.6	3.0	0.5	0.5
Diclofop-methyl	12.2	1.6	1.5	1.2	0.7	0.5	0.4	0.6	0.4	0.3
EL 5261	12.4	9.9*	4.6	3.9	2.5	2.6	1.5	1.4	0.6	0.3
EPTC	12.6	11.0	9.4	8.4	6.6	6.6	5.7	3.7	1.8	0
Ethalfluralin	12.4	8.4	5.6	3.0	2.6	2.2	2.8	1.8	0.8	0.4
Fluazifop-butyl	12.6	7.9	9.6	7.0	6.5	2.0	1.5	1.3	0.8	0
Glyphosate	11.6	10.7*	10.8*	10.7*′	11.6*	11.5*	10.5*	7.8	2.4	2.5
Metribuzin	11.6	10.6*	12.4*	12.4*	11.9*	11.4*	11.7*	9.9	1.0	0
Nitrofen	12.4	3.7	4.0	2.4	3.7	1.5	1.4	1.4	0.7	0
Sethoxydim	12.6	5.7	7.0	7.9	10.6	6.4	2.6	1.6	0.6	0.6
TCA	12.6	11.8*	11.5*	12.3*	11.3*	11.6*	10.9*	11.5*	12.1*	10.1
Trifluralin	12.6	10.3	10.7*	10.3	10.8	7.8	4.4	2.2	0.8	0.7

[a] Readings are the mean of three replicates maintained at 21°C in darkness for two days

[b] All comparisons of regression line slopes (cm/48 h) between 0 ppm (control) and respective herbicide concentrations are significantly negative unless denoted by * where there is no significant difference (P = 0.05)

Table 19.5.2 Total weight of sclerotia of *Sclerotinia sclerotiorum* per plate of potato dextrose agar amended with various concentrations of pre-or post-emergence herbicides (Adapted from the publication of Cerkauskas et al., 1986. With permission)

	Total sclerotial weight (mg) per herbicide concentration (μg a.i./ml)									
Herbicide	0	1	2.5	5	10	25	50	100	500	1,000
2,4-D ester	220a	13b	210	200	190	230	150	100–	60–	40–
2,4-DB	240	250	260	270	260	280	270	260	100–	50–
Alachlor	240	250	260	240	250	240	250	260	210–	240
Atrazine	210	210	220	170	150	170	180	180	210–	190
Barban	240	210	280+	280+	300+	260	160–	100–	0–	0–
Benazolin	210	250+	240	220	250+	250+	240+	270+	130–	130–
Bromoxynil	210	230	250	240	200	150–	190	110–	0–	0–
Cyanazine	220	250	240	270+	250	270+	250	240	180	180
Dalapon	220	240	220	240	220	200	220	200	190	200
Diallate	220	200	180	190	180	200	140–	140–	0–	30–
Diclofop-methyl	210	250+	260+	250+	250+	200	140–	100	70–	50–

(continued)

Table 19.5.2 (continued)

Herbicide	Total sclerotial weight (mg) per herbicide concentration (μg a.i./ml)									
	0	1	2.5	5	10	25	50	100	500	1,000
EL 5261	210	250	270+	240	250	250	260	210	200	90–
EPTC	220	210	170	200	200	200	220	220	120	110–
Ethalfluralin	210	250	260+	260+	260+	240	230	210	120–	80–
Fluazifop-butyl	220	160	200	220	230	190	190	160	70–	40–
Glyphosate	210	180	190	200	170	220	130–	130–	110–	110–
Metribuzin	210	190	220	240	170	180	240	210	110–	0–
Nitrofen	210	240	260+	250	230	210	200	190	100–	60–
Sethoxydim	220	190	190	200	150-	170	230	180	70–	80–
TCA	220	170	190	190	220	180	210	200	190	210
Trifluralin	220	180	170	160	150–	170	180	210	110–	50–

Table 19.5.3 Effect of EPTC, triallate and trifluralin on incidence of carpogenic germination and rotting of sclerotia of *Sclerotinia sclerotiorum* after incubation in a Sutherland clay loam soil for 120 days (Adapted from the publication of Cerkauskas et al., 1986. With permission)

Herbicide	Condition of sclerotia	Soil	Number per herbicide concentration (μg a.i./g soil[a])					Regression co-efficientc	Coefficient of deter-mination
			0	1	5	10	20		
EPTC	Healthy[b]	NA[c]	32[d]	25	22	27	18		
	Germinated		21	11	13	14	8	0.40	0.45
	Rotted		67	84	85	79	94	0.88	0.52
Triallate	Healthy	NA	41	24	33	30	22		
	Germinated		17	8	14	12	13	0.01	0.001
	Rotted		62	88	73	78	85	0.58	0.21
Trifluralin	Healthy	NA	23	29	29	36	18		
	Germinated		7	13	11	9	11	0.03	0.01
	Rotted		90	78	80	76	91	0.26	0.10
EPTC	Healthy	A	39	44	30	37	27		
	Germinated		15	25	20	23	19	0.02	0.004
	Rotted		66	59	55	47	51	0.69	0.55
Triallate	Healthy	A	37	36	45	50	50		
	Germinated		16	25	20	23	19	−0.02	0.004
	Rotted		67	59	55	47	51	−0.069	0.55
Trifluralin	Healthy	A	42	46	40	50	41		
	Germinated		14	17	14	19	12	0.11	0.11
	Rotted		64	57	66	51	67	0.15	0.03

[a] Air-dry basis

[b] Sclerotia maintained at 15°C and 80% R.H. and 6,500 lux after stipe emergence. Extraction from soil using water and 0.85 mm sieve underneath a 2.00 mm sieve and allowed to dry for one day. Intact = healthy

[c] NA and A denote non-autoclaved and autoclaved soil respectively

[d] Sum of eight replicates per herbicide concentration with 15 sclerotia per replicate

[e] No significant (P = 0.05) effects of herbicide concentration on carpogenic germination

apricots and almonds. Byrde (1952) found that dipping plums in sodium DNOC reduces the number of pustules caused by *S. laxa*. According to Marcano et al. (1983), the mycelial growth, production, characteristics of germination and viability of sclerotia of *S. sclerotiorum* are affected by different concentrations of the herbicides, Alachlor, Paraquat, Pendimethalin and Metribuzin when compared to control (PDA). Lazo (Alachlor) is more effective in reducing mycelial growth and production of sclerotia per plate. Dinoseb and Dyanap incorporated into PDA at 1 µg/ml significantly reduce mycelial growth of *S. minor*. Mycelial growth also is reduced on media containing 2, 4-D, B at 25 µg/ml. Field application of Dinoseb and Dyanap at 0.84 kg/ha significantly reduces the severity of *Sclerotinia* blight in peanuts when applied at post-emergence stage (Porter and Rud, 1980). However, an increase in stem rot of canola after application of Barban has been noted by Berkenkamp and Friesen (1973). According to Radke and Grau (1986), out of nine herbicides tested, trifluralin, pendimethalin, metribuzin, Simazine and Atrazine stimulate carpogenic germination of *S. sclerotiorum* as measured by the percentage of germinated sclerotia and by number of stipes and/or apothecia that develop per sclerotium. Linuron and DNBP inhibit germination and apothecium development and Alachlor causes variable responses. Simazine and Atrazine enhance stipe formation but stipes and apothecia are malformed. Metribuzin and Atrazine enhance stipe and apothecial growth without malformation. The herbicide Lactofen application in the soil @ 70 g/ha delays reproductive development, reduce the leaf area index, reduce *Sclerotinia* stem rot lesion diameter and increases phytoalexin production in soybean after 2–26 days after treatment (Nelson et al., 2002b). Glyphosate herbicide and shading does not affect the glyphosate resistant soybean defense response to *S. sclerotiorum* (Lee et al., 2005). All the herbicides tested by Pelmus et al. (1988) reduced mycelial growth, sclerotial formation and apothecial production, the maximum effect being shown by Dual (Metalachlor), Dizocab (butylate) and Eradicane (EPPC). Chlorsulfuron, Cyanazine, Metribuzin, Tri-allate and Trifluralin (mixed in top 8 cm of soil at 0.02, 1.0, 0.28, and 1.4 kg/ha respectively and double these rates) significantly reduces the carpogenic germination of sclerotia of *S. sclerotiorum* (Teo et al., 1992). According to Reichard et al. (1997), the fungicide Vinclozoline is more toxic to *S. trifoliorum* than any of the herbicide tested and it completely inhibits mycelial growth and ascospore germination at 1 µg a.i./ml. Herbicides Alachlor and Basalin causes 100 per cent inhibition of *S. sclerotiorum* mycelial growth. Plant extracts of *S. lappa* and *L. camara* are most toxic to this pathogen in pea (Sharma and Kapoor, 1999a).

19.6 Chemicals Effective Against Various Stages of the Pathogen

Out of nine fungicides tested by Hawthorne and Jarvis (1973), Benomyl, Dichlozoline and Thiram at 5 ppm caused at least 60 per cent inhibition of mycelial growth of both *S. minor* and *S. sclerotiorum*. Thiophanate and thiophanate-methyl are more active against *S. sclerotiorum*. At low concentrations (34 ppm), Captan,

Dichlofluanid and Thiram completely inhibit germination of ascospores and act as fungicidal. Germination of sclerotia of both fungi is completely inhibited at 10–100 ppm by all fungicides except Dichloran and quintozene. Benomyl, dichlozoline and thiophanate-methyl at 10^{-2} M completely inhibit stipe formation by sclerotia of both fungi. Quintozene inhibits stipe formation more in *S. sclerotiorum* than in *S. minor*, but the reverse is true for the other fungicides (Tables 19.6.1, 19.6.2; Figs. 19.6.1–19.6.4). Bavistin, Topsin M, Silaxyl –MZ, mancozeb and neem extract completely inhibits mycelial growth of *S. sclerotiorum* at conc. of 50, 100, 1,000 and 5,000 ppm respectively (Zewain et al., 2004). However, Singh et al. (2003) found carbendazim (25 and 50 µg/ml) and Ridomil MZ –72 (50 µg/ml) as most inhibitory for mycelial growth of *S. sclerotiorum*. Ridomil MZ and Kitazin at higher concentrations reduce sclerotial germination also. The presence of 62.5–125 µM of triadimefon in glucose nitrate liquid medium reduces mycelial growth and inhibits sclerotial formation of *S. sclerotiorum*. Hyphae grown in the presence of triadimefon contain more mitochondria and endoplasmic reticulum (ER). The additional ER occurs both as randomly oriented material and as membrane complexes or stacks. Membrane-bound vesicles accumulate between the plasma membrane and the cell walls (Stiers et al., 1980). Guanylurea sulphate is completely inhibitory for both stipe and apothecial formation after a 24–96 h treatment of cold conditioned sclerotia of *S. sclerotiorum* with an aqueous solution at a concentration of 10^{-3} mol/l (Finck and Bomer, 1985; Finck, 1989). Nearly the same effect is obtained by dicyandiamide at 10^{-2} mol/l. Fungicides like Vinclozolin, Iprodione, Thiram and Captan kill sclerotia of *S. sclerotiorum* in 45–180 min (Nedeleu et al., 1988).

Table 19.6.1 Fungicidal-fungistatic activity of fungicides against ascospores of *Sclerotinia minor* (isolate H10) (Adapted from the publication of Hawthorne and Jarvis, 1973. With permission)

	Ascospore germination[a] (%)			
	During exposure to fungicide		After exposure to fungicide	
Fungicide[b]	16 h	24 h	8 h	24 h
Benomyl	57	95	89	97
Captan	1	1	0	1
Dichlofluanid	0	1	0	0
Dichlozoline	4	1	50	96
Dicloran	60	66	70	[c]
Quintozene	31	80	89	[c]
Thiophanate	70	89	94	[c]
Thiophanate-methyl	66	94	89	[c]
Thiram	0	0	0	15
None	90	98		

[a] Ascospores were incubated on cellophane discs on the surface of the agar medium containing the fungicide

[b] All fungicide tested at a conc. 500 ppm in cornmeal agar

[c] Growth of the germ tubes was too extensive to permit an accurate reading

Table 19.6.2 Effect of fungicides on the formation of stipes from sclerotia of *Sclerotinia minor* and *Sclerotinia sclerotiorum* (Adapted from the publication of Hawthorne and Jarvis, 1973. With permission)

Fungicide (10^{-2} m)	Inhibition of formation of stipes (%)	
	S. minor[a]	*S. sclerotiorum*[b]
Benomyl	100	100
Captan	97	30
Dichlofluanid	92	3
Dichlozoline	100	100
Dicloran	96	83
Quintozene	70	100
Thiophanate	100	30
Thiophanate-methyl	100	99
Thiram	72	17

[a] Stipe formation measured after 11 week of incubation in the fungicide suspension; 76% of the sclerotia formed apothecial initials in the tap water (isolate H10)

[b] Stipe formation measured after eight week incubation in the fungicide suspension; 96% of the sclerotia formed apothecial initials in the tap water control (isolate H31)

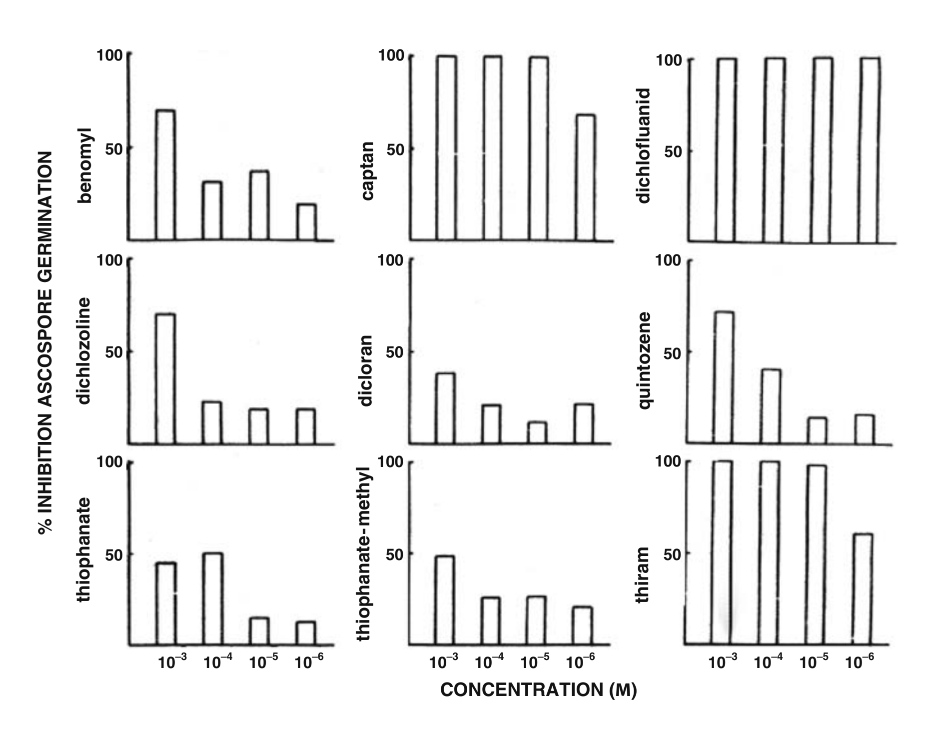


Fig. 19.6.1 Effect of fungicides on germination of ascospores of *Sclerotinia sclerotiorum*. Germination of ascospores in distilled water was 78 per cent (Adapted from the publication of Hawthorne and Jarvis, 1973. With permission)

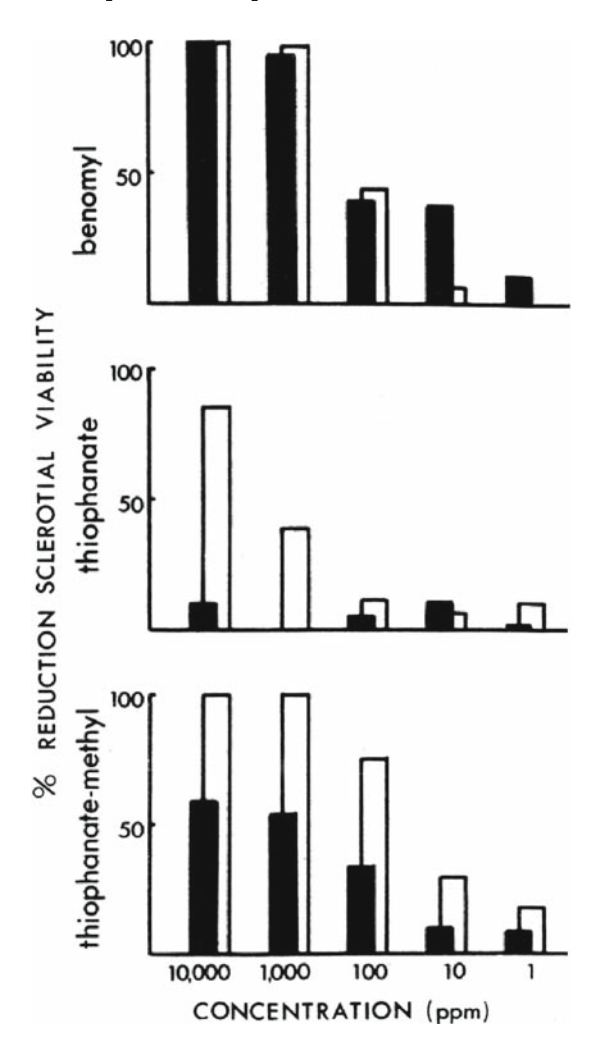


Fig. 19.6.2 Effect of a four days exposure to fungicide on sclerotial viability of *S. minor* (■) and *S. sclerotiorum* (□). Viability of sclerotia after four days in distilled water was 96 per cent for both *S. minor* and *S. sclerotiorum* (Adapted from the publication of Hawthorne and Jarvis, 1973. With permission)

The thyone oil at 10 ml, purple basil at 25 ml, manjerona at 25 ml, mint citrate at 50 ml, tarragon at 50 ml are capable of inhibiting the growth of *Sclerotinia* (Diniz et al., 2005). Application of nitrolim (57 per cent calcium cyanamide), Benlate and benzotriazole inhibits germination of sclerotia over a period of at least 20 weeks at 20°C (Jones and Gray, 1973). Calcium cyanamide and mylone (30–200 and 150–500 lb/acre) completely inhibit apothecial formation (McLean, 1958a). Vapam, Chloropicrin, methyl bromide and Mylone are effective in killing sclerotia in soil. With Vapam, drenching is best for killing sclerotia 1 and 5 cm deep. Lateral movement of Vapam is greater when the relative soil moisture content is high as 92.6 per cent (Partyka and Mai, 1962). 6H-3-phenyl-5-methyl 1-7-[3,4dichlorophenyl pyrazole [3,4-C](1,2,5) thiadiazine-2,2-dioxides at 200 mg/l completely inhibits *S. minor*

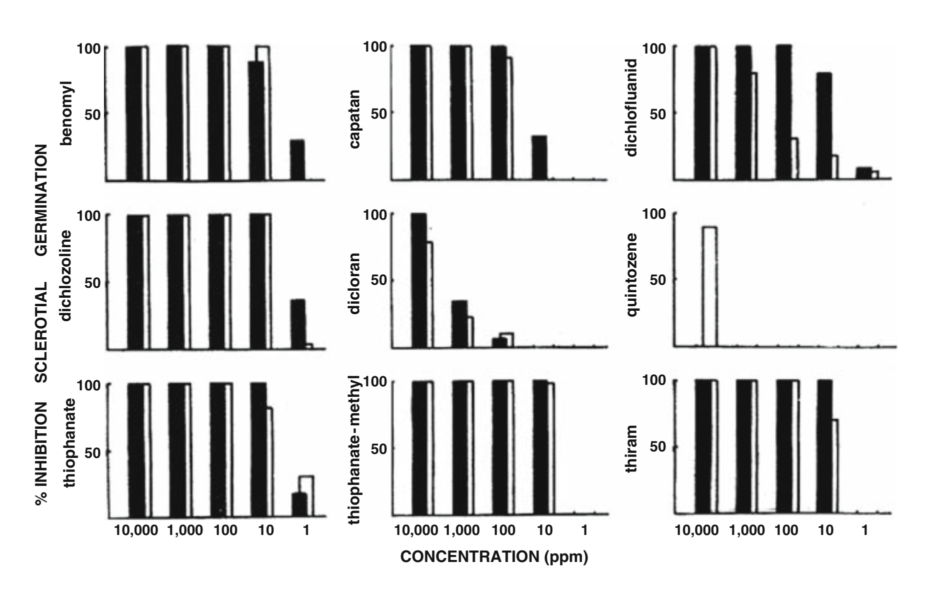


Fig. 19.6.3 Inhibition of sclerotial germination of *S. minor* (■) and *S. sclerotiorum* (□) after seven days in cornmeal agar containing fungicide. Sclerotial germination in control (no fungicide) was 94 and 98 per cent for *S. minor* and *S. sclerotiorum* respectively (Adapted from the publication of Hawthorne and Jarvis, 1973. With permission)

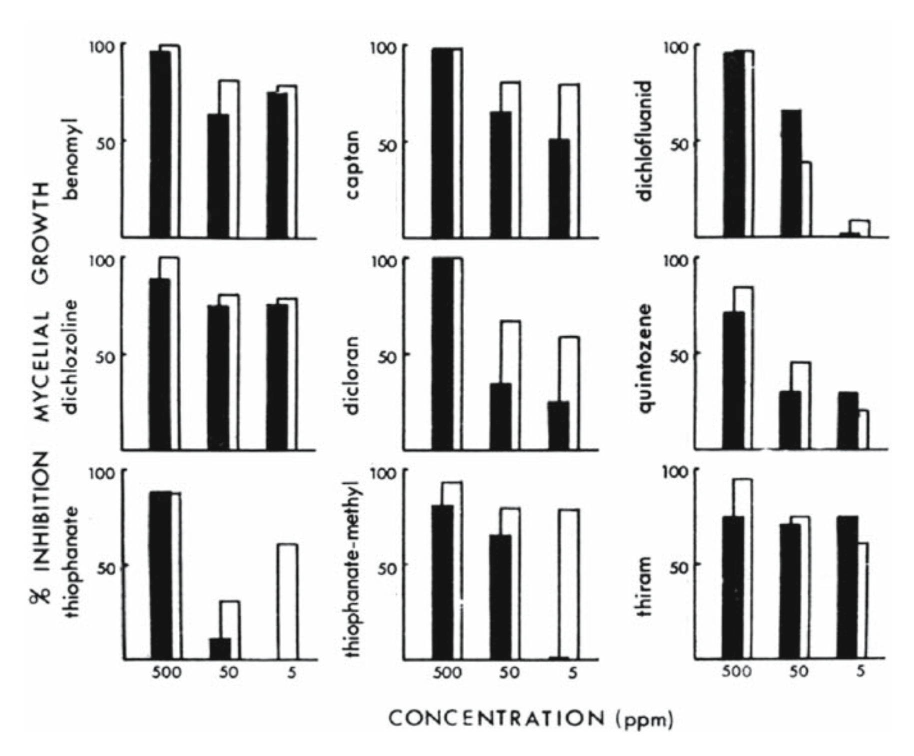


Fig. 19.6.4 Inhibition of mycelial growth of *S. minor* (■) and *S. sclerotiorum* (□) in liquid media containing fungicide. Dry weight of mycelium produced in control (no fungicide) was 88 mg for *S. minor* and 127 mg for *S. sclerotiorum* (Adapted from the publication of Hawthorne and Jarvis, 1973. With permission)

(Vicentini et al., 1990). Carbendazim at 25 μg/ml a.i. gives complete inhibition of mycelial growth on Richard's agar medium. No sclerotial production is observed in carbendazim (50 μg, 37 μg/ml), Captan (250 μg/ml) and Metalaxyl + Mancozeb (50 μg/ml). These fungicides also give 100 per cent inhibition of ascospore germination (Singh and Kapoor, 1996; Singh et al., 1996a).

LeTourneau (1976) analyzed effect of phenylthiourea on growth and sclerotial formation of *S. sclerotiorum*. The growth rate of *S. sclerotiorum* is reduced when 5×10^{-4} to $2–10^{-3}$ M I-phenyl-2-thiourea (PTV) is incorporated into synthetic media on PDA. The pathogen produces heavy aerial mycelia and few, if any sclerotia in synthetic glucose nitrate liquid medium containing 10^{-3} and 2×10^{-3} M PTV. At the same PTV concentration in PDA, it forms abnormal sclerotia covered with a yellowish green exudates. Steadman and Nickerson (1975) found compounds like divalent metal ions, simple sugars and common counterious, buffer inhibitory to sclerotia of *S. sclerotiorum* comparable with fungicides like Benomyl. Trevethick and Cooke (1971) concluded that chelators and enzyme inhibitors have an indirect effect on sclerotial formation by altering unrelated metabolic processes. Later, nutritional studies indicated that Zn may be necessary for the formation of sclerotia of *S. sclerotiorum* (Vega and LeTourneau, 1974).

19.7 Foliar Application of Fungicides

As with the prevention of most diseases, chemicals to control those caused by *Sclerotinia* spp. must be applied before infection occurs. Because many *Sclerotinia* diseases are initiated by colonization of senescent plant organs and the fungicide must be applied to prevent colonization of these organs. Proper timing of fungicidal sprays and adequate coverage of susceptible tissues of the host are crucial for obtaining effective control of *Sclerotinia* diseases with foliar applications.

19.7.1 Lettuce

In lettuce, myceliogenic germination of *S. minor* causes direct infection from soil. Thus, soil surface coverage near the plant and timing of fungicide application are the most important factors in obtaining control (Marcum et al., 1977). Soil treatment with Iprodione or Vinclozolin (at 100 g/m^3) should be followed after planting and depending on soil infestation, by two to three leaf sprays with Iprodione, Vinclozolin or Procymidene at 1.5 kg/ha before the 18 leaf stage (Davet and Martin, 1980). Benomyl (methyl 1-(butylcarbamoyl]-2 benzimidazolecarbamate), PCNB (pentachloronitrobenzene) and DCNA (2, 6-dichloro-4nitro aniline) are partially effective in California when applied as a single spray immediately after thinning (Marcum et al., 1977). In contrast, in Florida and New Jersey, multiple applications of Benomyl or DCNA are recommended every 10–14 days after transplanting to

minimize lettuce drop. Three applications of Ronilan (Vinclozolin) at 600 g/ha or Rovral (Iprodione) at 1,000 g/ha between planting and head development controls *S. sclerotiorum* as satisfactorily as the standard Benomyl treatment (Fischer, 1979; Matheron and Matejka, 1989). However, Davet and Martin (1979) recommended three foliar sprays with Iprodione or Vinclozolin, each at 750 g a.i./ha between the week after planting and the 18 leaf stage against collar rot of lettuce. The collar should be properly moistened, using the products at 1,000–1,500 l/ha. In heavily infested soil this should be supplemented by adding the fungicides at 5 g a.i./l001 while preparing composts. Matheron and Matejka (1989) have obtained control of *Sclerotinia* leaf drop by new fungicides like CGA-449, SC0858, SDS-65311, Bay HWG-160 g and Spotless (dinicomazole) equivalent to Vinclozolin and Iprodione. Use of antioxidants like gluconic acid lactone thiourea and propyl gallate reduces *S. sclerotiorum* on lettuce by 51–76 per cent (Elad, 1992).

Bottom rot of lettuce is controlled by the three mixture sprays of procymidone (30.8 g/1,000 m^2) + Thiram (122.5 g/1,000 m^2) or by soil drenching (1.1 kg + 4.4 kg/1,000 m^2) under Italy conditions (Minuto et al., 1999). In lettuce plots infested with *S. minor*, boscalid and fluazinam provides the highest level of disease control (Matheron and Porchas, 2004). One spraying of Sumiselex after thinning gives excellent control of *S. minor* in lettuce (Watson et al., 2002).

19.7.2 Beans

The growth habit or canopy density of a crop may influence the effectiveness of aerial application of fungicides against *S. sclerotiorum*. The poor deposition of Benomyl on the lower portions of bean plants may be due to the vigorous vine growth and thus responsible for lack of control. Botran at 0.9 kg a.i./acre provides economic control of white mould of beans applied at weekly intervals (Natti, 1967). Gabrielson et al. (1971) compared Botran with TBZ and Benlate and found that TBZ and Botran effectively controlled *S. sclerotiorum* on beans. However, Natti (1967) and McMillan (1973) found Benomyl and TBZ most effective for control of white mould of beans. In Florida, an aerial application of Benomyl combined with an earlier ground spray and an in-furrow treatment at planting gave excellent control of white mould on pole beans (Fig. 19.7.2.1), which have a more upright open canopy (Steadman, 1979, 1983).

Efficacy of fungicidal control of white mould of beans is determined by coverage of blossoms with a chemical such as Benomyl. Hunter et al. (1978) found that if the whole plant or only bean blossoms are sprayed with Benomyl, effective control results when plants subsequently are inoculated with a suspension of *S. sclerotiorum* ascospores. On the other hand, if all aboveground plant parts except blossoms are covered with Benomyl, no control is achieved. In Nebraska, when two applications of Benomyl are made to dry edible beans at first bloom and seven days later and just before canopy coverage precludes further ground applications. Where chemical control has been effective, timely blossom coverage probably has been

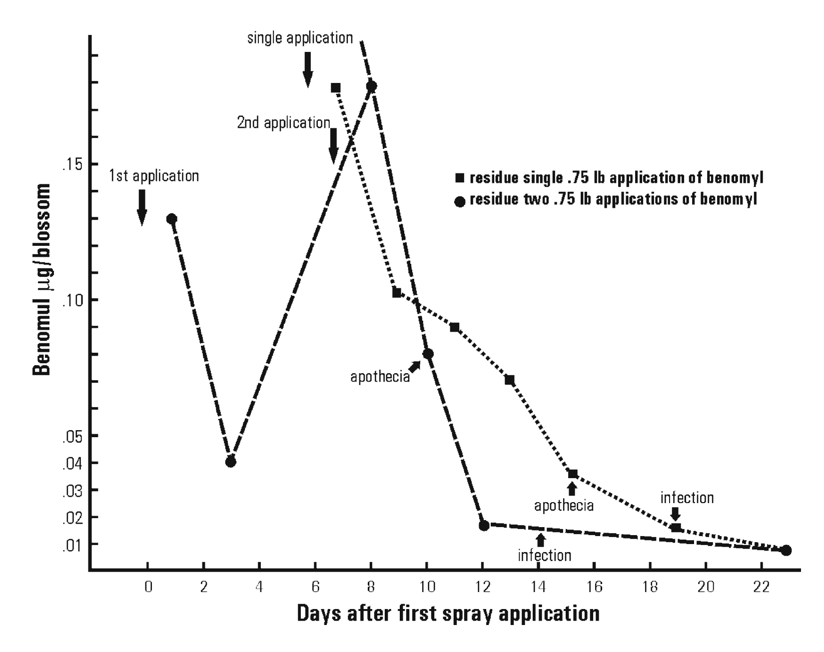


Fig. 19.7.2.1 Quantities of benomyl detected by bioassay in great northern bean blossoms until 23 days after single or double spray applications (Adapted from the publication of Steadman, 1983. With permission)

achieved (Steadman, 1979). One application of a fungicide such as Benomyl, 2, 6-dichloro-4-nitroaniline (DCNA) or Thiophanate-methyl (Topsin M) can be economical if disease reduction is satisfactory (Steadman, 1983). However, green bean white mould has been effectively controlled by spraying Benlate at 2.24 kg/ha (50 per cent formulation). Acceptable control can apparently be achieved with one spray applied three to seven days before full bloom. For good control, two sprays should be applied one at pre-bloom and the other at full bloom stage. Good coverage is important and wetting agent should be used (Natti, 1971; Wong, 1978). According to Sherf and Macnab (1986) for single spray programme, benomyl (350 g a.i./acre or even better, two sprays using benomyl (225 g a.i./acre) give good control. Timing is critical with the first application made when 75 per cent of the plants show an open blossom and the second applied seven days later. This ensures benomyl concentration in the blossoms at all times. Application before bloom or at the pin pod stage probably is wasted. Ideal application involves a ground sprayer with one nozzle over the row and drop nozzles between the rows, the lower portion of the plant and the soil surface around it must be covered. Aircraft application may result in erratic control because of inability to reach these areas. Although benomyl and thiophanate-methyl (Topsin-M) are the fungicides of choice, Dichloran, Dichlone, PCNB, Iprodione and Thiobendazol have been used with some success. According to Baraer (1979), on bean, Ronilan (Vinclozolin) applied twice at the beginning of flowering and when the first pods are 4 cm long, or once only at the

height of flowering gives excellent protection with increase in yield. Benoist (1979) recommended for French bean (green type), one treatment with Ronilan (Vinclozolin) at 1.5 kg/ha in 1,000 l when the first flower opens and for shelling type, two treatments at the same dose when the first flower opens and 20 days later.

The effects of volume, timing and number of sprays of Benomyl and arrangements of nozzles on the efficacy of chemical control of white mould of white bean have been determined by Morton and Hall (1989). The greatest suppression of disease and the greatest increase in seed yield are obtained when fungicides are applied at full bloom at a dose of 1.1 kg a.i./ha in 550 l water/ha through three flat fan nozzles per row, one nozzle on a horizontal boom, above the row and held by skid booms 8 cm above the soil surface to direct spray at the base of the plants. Disease control is less effective when the volume of spray is reduced from 550 to 243 l/ha and when nozzles are arranged on a horizontal boom and drop arms or on a horizontal boom only. The level of disease control does not depend on the time of spraying during flowering but is directly related to the number of blossoms within the canopy that receive fungicide. To control bean *Sclerotinia*, benomyl (0.5 kg a.i./ha), benomyl + mancozeb (0.5 + 1.6 kg a.i./ha), benomyl + iprodione (0.5 + 0.37 kg a.i./ha), iprodione (0.75 kg a.i./ha) and thiophanate methyl (1.15 kg a.i./ha) have higher efficiency when applied through water irrigation (Oliveira et al., 1995).

In fields with a history of white mold, apply fungicide at 1–10 per cent bloom (i.e., 1–10 per cent of the plants in the field have at-least one open bloom). A second application may be necessary with highly susceptible cultivars or heavy disease pressure.

1. Endura at 200–275 g/acre. The use of a non-phytotoxic adjuvant may improve the performance of Endura. To limit the potential for development of resistance, do not make more than two applications of Endura per season. The pre-harvest interval is seven days.
2. Rovral 4F at 1.5–2 pt/acre in at least 250 l/acre water. Not recommended in Idaho due to lower level of control. Apply at 1–10 per cent bloom and again five to seven days later or no later than peak bloom. No more than two applications per season. Crops that may be rotated after harvest with garlic, dry bulb onion, broccoli, lettuce, carrot and potato. Crops that may be rotated one month after last treatment with root crops and tomatoes. Do not allow foraging for 14 days after last application. Do not feed dry bean hay to livestock until 45 days after last application.
3. Topsin 4.5 FL at 30–40 fl oz/A for one application or 20–30 fl oz/A for two applications. For one application, apply when 100 per cent of plants have at-least one bloom open or when conditions are favorable for disease development. For two applications, make the first application when 10–30 per cent of the plants have at-least one bloom open and repeat application four to seven days later. Do not apply more than 80 fl oz/acre /season. Pre-harvest interval is 14 days.
4. Topsin M 70WP at 400–600 g/acre with first application at 10–30 per cent full bloom and a second application seven days later, or Topsin M 70WP at 600–800 g/acre with a single application at 50–70 per cent full bloom. Do not apply within 14 days of harvest. Do not use if the crop is for forage. May be applied through sprinkler irrigation lines according to label directions.

5. Botran 75W at 900 g/acre for bush variety or 1.6 kg/acre for pole variety on seven day intervals. Use in the past has shown poor efficacy.
6. Ronilan EG is no longer registered on snap beans

19.7.3 Rapeseed-Mustard

In India, white rot of mustard can be effectively controlled with three foliar sprays of benomyl at 0.025 per cent followed by Ziram (0.156 per cent) with increase in seed yield (Roy and Saikia, 1976). A significant reduction in disease development and lesion size has been observed by Dhawan (1980) with the spray of Benlate and Topsin-M in *B. juncea*.

In Europe, spray application of prothioconazole 250 EC (Proline) at the rate of 175 g a.i./ha controls *S. sclerotiorum* in oilseed rape/canola (Davies and Muncey, 2004). In Canada, a single aerial spray application of Benomyl at the early bloom stage in the disease-prone regions is suggested (Morrall and Dueck, 1983). A single application of the fungicides Benomyl and Vinclozolin effectively controls *Sclerotinia* stem rot in rapeseed when applied at 25 per cent bloom. Control is less consistent with Iprodione. Application of Benomyl at 1.0 kg and Iprodione at 0.5 kg a.i./ha by aircraft in large scale tests provides control equivalent to comparable treatments in small plot tests (Dueck et al., 1983). Aerial application of Benomyl to the rapeseed cvs. Altex and Candle reduces the level of *Sclerotinia* stem rot from 44 to 8 per cent with increase in yield and this application is economically feasible when yield is increased by at least 180 kg/ha (Thompson et al., 1984). Morrall et al. (1989) found Benomyl better than Iprodione since efficacy of Benomyl can be improved by selecting nozzles and spray pressures to produce small sized spray droplets thus enabling low volumes of carrier fluid and reduced dose rate of the fungicide. Moons (1986) found Botran FL (4 kg a.i./ha), Benlate (0.75 kg a.i./ha) and Gacazatine (0.75 kg a.i./ha) very effective against stem rot of canola. However, Shen (1992) found 4 per cent carbendazim (100 m1/M.W.) sprayed once at the middle of flowering effective against rape rot. Spraying against stem rot with Vinclozolin during full flowering increases the yield (Nordin et al., 1992). According to Tewari and Conn (1992), foliar application of calcium carbonate, calcium nitrate and calcium chloride significantly reduces the pathogenesis of *S. sclerotiorum* on canola. Calcium nitrate and calcium chloride are effective at much lower levels than calcium carbonate. These are soluble in water which may be an important factor in so far as field application is concerned. In Canada and the USA, spray application of prothioconazole 480 EC in rape/canola is recommended @ 150–200 g a.i./ha for effective control of *S. sclerotiorum* (Davies and Muncey, 2004). However, Bradly et al. (2006) reported that fungicides like azoxysrrobin, benomyl, boscalid, iprodione, prothioconazole, tebuconazole, thiophanate-methyl, trifloxystrobin and vinclozolin consistently reduces *Sclerotinia* stem rot of canola.

In Poland, Contans WG (*C. minitans*) used before sowing, while Alert 375 SC (Flusilazole + carbendazim) used during flowering of oilseed rape decreases *S. sclerotiorum* infection and increases yield (Weber, 2002).

In Germany, Kruger (1973) recommended application of 500 kg/ha calcium cyanamide in the spring to destroy sclerotia in the field along with weeds without any injury to rapeseed. Whereas Finck (1989) found transformation products of calcium cyanide like dicynamide and guangl urea sulphate as strong inhibitors of sclerotial germination and apothecial formation if applied in soil. Application of Vinclozolin at 1.5 kg/ha at full flowering stage has been found very effective for the control of white stalk rot on rape with increase in yield up to 8 per cent (Kruger and Stoltenberg, 1983). Saur (1983) suggested use of spore traps for optimal timing of Vinclozolin applications to get maximum disease control. The use of Iprodione, Prochloraz + carbendazim and tebuconazole (Flicur) sprays on rapeseed gives good control of disease with increase in yield (Paul and Beineke, 1993). The optimum period for chemical control of *Sclerotinia* disease of rape is between 100 per cent flowering stems and 80 per cent flowering branches. Second spray should be applied five to seven days after the first spray when all the branches are flowering (Zhang Xi Lin, 1998).

In France, fungicides viz., Benomyl (1,000 g a.i./ha), Procymidone, Vinclozolin, Iprodione WP and Iprodione flow at 750 g a.i./ha have been found effective against *Sclerotinia* disease of rapeseed. However, Vinclozolin and Procymidone are most effective (Regnault and Pierre, 1984). Flutriafol (117.5 g a.i./ha) + carbendazim (250 g a.i./ha) gives good control of stem rot of rape with increase in yield in U.K. and France (Noon et al., 1988). However, Cameron et al. (1986) found Guazatine as an effective fungicide against *S. sclerotiorum* on oilseed rape.

In Switzerland, the best control of *S. sclerotiorum* is achieved with a fungicidal spray at full flowering. Sclerotial germination is reduced by calcium cyanamide applied in early spring when new shoots are 5–10 cm long. Prophylactic measures include the use of less susceptible cvs. crop rotation, careful under ploughing of diseased plant debris, control of the cabbage stem weevil, control of volunteer rape and cruciferous catch crops and cruciferous weeds including shepherd's purse and wild mustard (Winter et al., 1993).

In China, an agricultural antibiotic 2–16 (at 100x and 150x dilutions) reduces *Sclerotinia* rot of rape by 82.6 and 78.1 per cent respectively (Xie-Chang Ju et al., 1999).

19.7.4 Peanut

In peanut, Botran, Benomyl and PCNB provide partial control of the disease (Beute et al., 1975). However, a single application of 4.48 kg/ha, 2, 6-dichloro-4-nitro aniline (DCNA) controls *Sclerotinia* blight of peanut in North Carolina (Beute et al., 1975). Foliar application of Procymidone (0.56 kg a.i./ha) has been found effective in the control of the disease (Porter, 1980c). Development of the disease is also

known to be suppressed by foliar sprays of copper or zinc sulphate when applied at 1.12 kg/ha (Hallock and Porter, 1979, 1981). Application of Iprodione with adjuvants like pinolene gives good control of *Sclerotinia* blight of peanut with increase in yield. Iprodione can be applied at 1.12 kg/ha + pinoline at 0.42 per cent in low volume sprays and 0.18 per cent (VN) in high volume sprays (Smith et al., 1991a). The application of Fluazinam @ 0.56 kg/ha (ASC-66825 and RH-3486) reduces disease incidence and increases yield (1,598 kg/ha) of peanut (Smith et al., 1992). Two or three sprays of Fluazinam @ 0.75 and 1.0 kg a.i./ha and of procymidone @ 0.688 and 0.75 kg a.i./ha are the most effective combinations that reduces disease incidence and increases yield of peanut (Ryley et al., 2000).

19.7.5 Sunflower

In sunflower, benomyl sprays have been found to control the disease. Two sprays of benomyl at the beginning of budding and at the end of budding or at early flowering give a better degree of disease control. Some other fungicides as thiophanate-methyl, mancozeb and Dichloran are also effective in controlling the disease (Acuna et al., 1976; Mackiewicz and Zub, 1982; Sharifi-Tehrani, 1974). The best effect with Vinclozolin in controlling *S. sclerotiorum* in sunflower is obtained with a single application of 0.75 kg a.i./ha at full flowering. However, two sprays, one at the beginning and one at the end of flowering provides better control (Hampel et al., 1981). Early treatments give significantly better control than later treatments. Fungicides like Konker (carbendazim + vinclozolin) at 1.5 and 2 kg/ha, mancozeb at 4 kg/ha, Kolfugo 25FW (carbendazim) at 1 kg/ha and Sportak PF (prochloraz) at 1.5 kg/ha are the most effective (Farady, 1988). Aceton and carbon tetrachloride (CTC) allow greater translocation of carbendazim within the plant for efficient control of seed and soil borne inoculum when used as organic solvent against *Sclerotinia* rot of sunflower (Kishore and Gupta, 1997). Alister and Trotus (1989) found fungicides like Apron SD-35 (metalaxyl), Ronilan (vinclozolin), Netratate, Rovral (iprodione) and Topsin M-70 (thiophanate-methyl) good for the control of *S. sclerotiorum* with increase in yield. According to Wu (1991), benomyl and DCNA (dichloran) controls *Sclerotinia* rot of sunflower in the field. Vinclozolin is more effective than dichloran or iprodione against the sunflower disease and inhibits ascospore germination completely. Shindrova et al. (1990) found Ronilan (vinclozolin) and Rovral TS (carbendazim + iprodione) very effective against *S. sclerotiorum.*

In Romania, thiophanate-methyl and vinclozolin give good control of *S. sclerotiorum* on hybrids Romsun-53 and Select with higher yields (Lazar et al., 1989). According to Huang (1992), in China, Ronilaas (Ronilan, vinclozolin) and Sumilex (procymidone) diluted to 1:500 gives best control of *S. sclerotiorum* on sunflower followed by carbendazim, thiophanate applied alone or in a 1:1mixture. In France, Konker (carbendazim 165 g/l + vinclozolin 250 g/l) gives best control closely followed by Bavistin (Guenin, 1991). In Yugoslavia, combination of vinclozolin, procymidone and iprodione with systemic fungicide carbendazim during flowering

controls *Sclerotinia* (Markovic, 1992). The following is a summary of sunflower disease management strategy.

- Sunflower should not be planted on land already infested with sclerotia. Fields that has been planted to susceptible crops such as dry bean or soybean can be infested, especially if in an area where *Sclerotinia* is common.
- Planting certified seed minimizes the danger of introducing sclerotia into fields that are free of *Sclerotinia*. Avoid solid seeding and high plant populations.
- Fields of susceptible crops should be monitored for disease incidence. Sunflower fields should be scouted about four weeks after flowering to assess incidence of wilt. A later date is even better, but as dry-down proceeds; it becomes harder to evaluate infected plants. A final scouting should occur prior to harvest to assess incidence of middle stalk rot and head rot. In other susceptible crops such as dry bean, it may be necessary to carefully search beneath the canopy to see sclerotia being formed. Accurate records of disease incidence and crop rotations are necessary for managing this pathogen.
- Rotations to a non-susceptible crop such as small grains, corn or sorghum are necessary when disease appears. Crop rotation is the most important management procedure. The rotation interval will depend upon disease incidence. A three to five-year rotation may be necessary with low disease incidence (less than 10 per cent), while six to eight years or longer might be needed at higher disease incidence. A dry land field with 10 per cent wilted sunflower plants might require a four to five year rotation to non susceptible crops to reduce the incidence of wilt to about 5 per cent. The incidence of wilt should not be permitted to exceed 1–2 per cent before starting a rotation to non-host crops. A low incidence of wilt increases substantially after several years of continuous sunflower and long rotations are then needed when there is a high level of sclerotia. Broad leaf weeds should be controlled.
- If sunflower is to be planted on *Sclerotinia* infested soils, choose the least susceptible commercial hybrids available.
- Sunflower should not be planted adjacent to a field infested the previous year because this may serve as a source of ascospores for head and middle stalk rots. (http:/ www. larry. chandler @ars. usda.).

19.7.6 Soybean

One application of benomyl (Benlate 50WP at 1 kg/ha) reduces *Sclerotinia* stem rot. Fungicide must be applied when soybeans are producing flowers or as pods are just emerging on the lower one half of the plant. Thus timing and penetration of the fungicides through the soybean canopy present problems in their effective use for control of *Sclerotinia* stem rot (Grau, 1988). Sumilex 50 WP (procymidone) and Trichosemin 25 PTS (*T. viride* 25 per cent) are the best to control *Sclerotinia* on soybean (Eva, 2003). Two applications of thiophanate – methyl starting at R_1 or at

the R_2 growth stage and a second application at two weeks later lowers the *Sclerotinia* rot of soybean along with seed borne inoculum (Mueller et al., 2004).

19.7.7 Forage Legumes

Clover canker caused by *S. trifoliorum* can be controlled by 0.12 per cent of benomyl and Fundazol (Mazur and Grechina, 1980). In alfalfa, Ronilan 50W (vinclozolin) provides excellent disease control caused by *S. trifoliorum*. Benlate 50W (benomyl), Botran 75W (dichloran), Kocide 101 (copper hydroxide), Rovral 50W (iprodione) and Topsin M 70W (thiophanate-methyl) are other effective fungicides. Application of vinclozolin at apothecium emergence reduces the number of lucerne and red clover plants infected by *S. trifoliorum* and increases dry matter yield as compared with an untreated control. Four sprays of vinclozolin are only 10 per cent more effective than the single application at apothecium emergence (Rhodes et al., 1992). However, four sprays of vinclozolin effectively controls crown and stem rot in alfalfa (Sulc and Rhodes, 1997).

In France, procymidone, vinclozolin and carbendazim + vinclozolin gives good disease control if applied in November especially on the young stands sown in the autumn at 1.5 kg/ha (Raynal et al., 1991).

19.7.8 Cabbage and Cauliflower

Numerous fungicides have been reported to reduce disease incidence with increase in yield, but Benomyl and Carbendazim are best. Benomyl is most cost effective (Sharma and Sharma, 1984b). Gabrielson et al. (1973) reported that cabbage seed crops can be protected with benomyl if stem surface coverage of the upright open canopy is adequate.

Curd rot of cauliflower can be controlled when the curds are pasted with a slurry of chloramphenicol + Captafol (1:25) and subsequently sprayed with an aqueous suspension of this mixture at 0.01 + 0.25 per cent during the season (Chakrabarty, 1993). *Sclerotinia* head rot of cabbage can be effectively controlled after spraying the fungicides like BWCO 14 F, BWCO 1201F, CGA 173506 (fludioxanil), fluazinam, ICIA 550 4 (azoxystrobin), Rovral (iprodione), Ronilan (vinclozolin) and Topsin M (thiophanate methyl) (Cubeta et al., 1998b).

19.7.9 Cucurbits

On melon, Ronilan (50 per cent vinclozolin), applied at 0.15 kg/ha and after flowering and 15 days before harvest reduces incidence of *S. sclerotiorum* by 75–80

per cent. On cucumber in the glasshouse, three applications at 0.1 kg/ha even 10–14 days from the first appearance of symptoms give 72 per cent protection against fruit rot (Anon., 1979). Benlate (benomyl) gives 91.42 per cent and Enovit (thiophanate methyl) gives 86.04 per cent control against *Sclerotinia* rot of cucumber (Tanas, 2004).

19.7.10 Tomato

Procymidone 0.5 kg/ha provides better control of *Sclerotinia* with increase in yield when compared to benomyl (Jackson and Smith, 1979).

19.7.11 Carrot

Pre-harvest and pre-storage fungicide applications can effectively decrease the viability of sclerotia in soil or reduce level of *Sclerotinia* rot of carrot (SRC) in the field and storage respectively. Foliar sprays with 0.3 per cent compass (167 g each of iprodione and thiophanate-methyl per litre), 13 and 17 weeks after seeding are effective in decreasing the level of SRC in the field and increasing marketable yield of carrots in Scotland (Couper, 2001). Reduction of disease development in stored carrots has been obtained by either two foliar applications of vinclozolin (Ronilan FL 500 g l^{-1} or 0.421 a.i. ha^{-1}), one before canopy enclosure and another in midseason, or by a single foliar application of vinclozolin at 0.841 a.i. ha^{-1} or benomyl (Benlate 50 per cent WP) at 1.0 g ha^{-1} ten days prior to harvest (Pritchard et al., 1992). One foliar application of thiophanate-methyl (Topsin–M 70 WP), the day before harvest completely controls infection by *Sclerotinia* in storage (Tahvonen, 1985). Effective control of SRC and subsequent reduction in crop losses during long term storage also have been achieved by pre-storage dip treatment of carrots in 0.1 per cent aqueous solution of sodium orthophenylphenate (Hoadley, 1963). Benomyl suspension 0.05 or 0.025 per cent a.i. or 0.05 per cent a.i. suspension of iprodione (Rovral 50 per cent WP) also effective in managing the disease (Cheah et al., 1997; Geeson et al., 1988).

19.7.12 Potato

The disease must be severe for fungicide application to be economically effective

1. Blocker 4 F at 3–10 pt/acre by ground or through irrigation at the first sign of disease or no later than just before row closure. Do not apply by air. Can reapply at seven to ten days intervals. Do not apply within 45 days of harvest, shorter pre-harvest interval with reduced rates.

2. Botran 5F at 1.2–3.6 qt/acre. Begin treatment at first flower drop, or at approximately the time of full row closure. If disease persists, subsequent applications may be made at seven to ten day intervals. Chemigation is the preferred method of application.
3. Apply as a directed spray to the base of plants and adjacent soil surface using drop nozzles. Use at-least 135 l/acre water. Do not spray upper canopy of foliage as this may cause minor leaf bronzing. If canopy is to be sprayed, use at-least 450 l water. Do not apply within 20 days of harvest.
4. Endura at 140–250 g/acre prior to row closure or at the onset of disease. Make a second application 14 days later if conditions continue to be favorable for disease development. Use the higher rates, once disease has been confirmed in your area or weather conditions are conducive to disease development. The use of a non-phytotoxic adjuvant may improve the performance of Endura. To limit the potential for development of resistance, do not make more than two applications of Endura per season. Pre-harvest interval is 30 days.
5. Omega 500F at 5.5–8 fl oz/acre at seven to ten-day intervals beginning when plants are 8 in. tall. Do not apply more than 3.5 pt/acre/season or within 14 days of harvest.
6. Rovral 4F at 2 pt/acre in at-least 450 l/acre water. Do not apply by air for white mold control. Apply at first sign of disease or just before row closure and again 21–28 days later. (These applications also control early blight.) Do not irrigate for 24 h after application. Do not apply within 14 days of harvest or more than four times in a season.
7. Topsin M WSB at 400–600 g/acre at 7–14 day intervals. Make first application just before row closure. Thorough coverage of the lower stems and branches is essential for disease control. Do not apply more than 1.6 kg product (1,120 g a.i.)/acre/season. Do not apply within 21 days of harvest. May be tank mixed with other fungicides labeled for early and late blight control. Aerial application is not recommended for control of this disease on this crop (http://www.pototatodiseases.org).

19.8 Post Harvest Disease Control

The post harvest dip of Botran at 800 g/100 l provides excellent control of nesting of pole beans (McMillan, 1969). Post harvest dips of bean pods give control of the post harvest phase of white mould. Dips in 125°F water alone for 30 s or with 450 ppm dichloran, 10 s dip in 1,140 ppm thiobendazole, or 10 s dip in 125°F thiobendazole (1,140 ppm) or dichloran (225 ppm) is effective for better management of the disease (Sherf and Macnab, 1986). Effective control of brown rot on stone fruit, both at harvest and in storage is attained with procymidone 30 g/100 l and iprodione 40 g/100 l. All these treatments are superior to Benomyl 20 g/100 l (Jackson and Smith, 1979). Diseases caused by *Sclerotinia* spp can be suppressed by controlled atmosphere storage at 0–1°C (7.5 per cent CO/1.5 per cent O_2, 1.5 per cent O_2, 4 per cent CO_2/1.5 per cent O_2). Watery soft rot of celery caused by

S. sclerotiorum is severe if stored in normal air for two weeks at 8°C whereas a comparable severity is attained after ten weeks at 1°C (Reyes, 1988). According to Afek and Carmeli (1995), GA-3 retards celery decay during storage by slowing down the conversion of (+) marmesin to psoralens, thereby increasing the resistance to pathogens during storage. *Sclerotinia* rot in carrot reduces during long term storage at 0°C by dipping the roots after harvest in a conidial spore suspension of *T. harzianum* (Tronsmo, 1989). Vinclozolin at 0.841 a.i./ha applied ten days before harvest significantly reduces cottony soft rot of carrot in storage (Pritchard et al., 1992). Carrot roots treated (coated) with chitosan solution at 2–4 per cent reduces significantly the incidence of *Sclerotinia* rot in storage (Cheah et al., 1997). Ozone at 60 µl/l reduces *S. sclerotiorum* on carrot during storage (Liew and Prange, 1994). An ozone supply of 15 µl $liter^{-1}$ for 8 h daily at 2°C is suggested for providing adequate disease control while preserving carrot quality (Liew and Prange, 1994). Optimum storage conditions and proper washing and grading of harvested carrots can substantially enhance the efficacy of pre-storage fungicide dip treatments (Geeson et al., 1988; Lockhart and Delbridge, 1972). Pre-storage dip treatment for 5 min in a conidial suspension (1×10^7/ml) of a cold tolerant mutant of *T. harzianum* reduces SRC severity during long-term storage at 0°C (Tronsmo, 1989).

Oils from dill, *Foenicum vulgare*, anise and *Majorana hortensis (Origanum majorana)* are particularly active against *S. sclerotiorum*. These oils do not affect the taste of vegetables and fruits treated (Crisan et al., 1978).

The possibility of controlling stem rot and wilt of gram caused by S. *sclerotiorum* has been suggested by Singh and Singh (1984b) through the extracts of ginger.

19.9 Biological Control

Several antagonistic microorganisms (fungi, bacteria, actinomycetes, yeast, algae, fungus gnat) have been reported to decrease the pathogenic activity of *Sclerotinia sclerotiorum* (Table 19.9.1). The suppression of pathogen by antagonists can occur in various ways, i.e., parasitism, predation, competition for nutrients and space, mechanical obstruction and production of toxic and inhibitory metabolites. Studies carried out by Hoes and Huang (1975) and Hoes (1977) indicate that *Coniothyrium minitans; Gliocladium catenulatum* and *Trichoderma viride* are frequently associated with sclerotia of *S. sclerotiorum* in Manitoba sunflower fields and about 50 per cent sclerotia are reported to be non viable. *C. minitans* is reported to be the most effective antagonist and field trials conducted over a period of three years have indicated that the introduction of *C. minitans* into *S. sclerotiorum* infested soil by seeding time decreases *Sclerotinia* wilt of sunflower, thereby reducing yield losses. Reduction of the disease is due to the effective control of primary inoculum or sclerotia by the hyperparasites (Huang, 1980; Turner and Tribe, 1976). It is interesting that *C. minitans* is more effective in parasitizing sclerotia produced on or inside the root than those produced in the basal stem. It is also reported that *S. sclerotiorum*

does not escape the antagonism even after it invades sunflowers because sclerotia formed inside the pith cavities of roots and basal stems are readily parasitized and subsequently killed by *C. minitans* (Hoes, 1977). This hyperparasite thus appears to be a promising biological control agent for *Sclerotinia* rot of sunflower (Turner and Tribe, 1976).

It is important that air dried *C. minitans* can control *Sclerotinia* wilt as effectively as the freshly prepared moist inoculum. This finding is of practical importance because it facilitates the incorporation of *C. minitans* into soil by mechanical means, which is necessary for large field scale testing or for commercial application (Huang, 1980). *Coniothyrium minitans* applied to soil as maize meal perlite in rape infected sclerotia of *S. sclerotiorum*, decreases sclerotial survival, carpogenic germination and production of apothecia (McQuilken et al., 1995; Huang and Ericksen, 2004).

Coniothyrium minitans and *Gliocladium virens* applied separately as solid substrate inocula to soil before planting, significantly reduces infection of lettuce caused by *S. sclerotiorum*. Spore sprays of the antagonist applied to crop residues significantly reduces infection in a subsequent crop. *C. minitans* results in a reduction in the number and viability of sclerotia (Budge et al., 1995).

Coniothyrium minitans A 69 when applied as a maize/perlite formulation to plots infected with sclerotia of *S. minor* six weeks prior to planting, disease control is effective upto 75 per cent (Ridgway et al., 2001; Stewart et al., 2001). *Coniothyrium minitans* isolate Conio applied as maize meal perlite solid substrate soil incorporation @ 10^{12} cfu m^{-2} gives significant control of *Sclerotinia* disease of lettuce equal to the fungicide Iprodione. This treatment also results in a reduction in the number and viability of sclerotia and increases infection of sclerotia by *C. minitans* (Jones et al., 2001). *Coniothyrium minitans* has potential as a biological control agent of *S. sclerotiorum* in western Canada because of its ability to over winter and become active after the winter (Huang and Erickson, 2002). Pre-germinated conidia of *C. minitans* enhance its efficiency significantly. In oilseed rape, hyphal extension of *S. sclerotiorum* is inhibited by 68 per cent while formation of sclerotia is completely inhibited when pre-germinated conidia are applied (Shi Jun Ling et al., 2004).

In Alberta, Canada, the application of *C. minitans* to soil at seeding time reduces apothecial production of sclerotia of *S. sclerotiorum* under the canopies of bean, canola, wheat and barley. Application of *Talaromyces flavus* is ineffective and combinations of *T. flavus* and *C. minitans* are as effective as or less effective than *C. minitans* alone, indicating that no synergism occurs between these hyperparasites. Application of *C. minitans* to soil in the spring reduces apothecial production from sclerotia of *S. sclerotiorum* buried in the soil and increases parasitism on sclerotia produced on bean plants (McLaren et al., 1996). Addition of biological control agents such as *C. minitans*, *P. griseofulvum* or *T. virens* enhances the suppressive effect of the organic soil amendments on *S. sclerotiorum* (Huang et al., 2005a). Recently ready for use formulations of a German isolate of *C. minitans* has been developed and it is now registered for use in *Sclerotinia* spp. susceptible crops in several countries. The application of *C. minitans* strain CON/M/91-08 effectively reduces the viability of the sclerotia in the soil (Aertsens and Michi, 2004).

Trutmann et al. (1983) proposed a strategy which includes the use of fungicidal sprays during the growing season and the application of *C. minitans* spore suspensions to slash crop debris containing sclerotia, which is subsequently buried by deep ploughing. The optimum temperature for germination, growth, infection of sclerotia and destructive parasitism by *C. minitans* is 20°C with >95 per cent relative humidity. However, in Australia, autumn inoculation with suspensions of conidia, pycnidia and mycelium of *C. minitans* in the field resulted in negligible numbers of sclerotia remaining viable after one month (Trutmann et al., 1980). Soil mesofauna like mite *Acarus sire* and the collembolan *Folsomia candida* transmits *C. minitans* atleast 55 mm to sclerotia in soil at water potentials ranging from saturation to −3.6 MPa. Soil mesofauna may be important in the dissemination of *C. minitans* (Williams et al., 1998d).

Trichoderma sp. when applied to seed and soil has been reported to reduce the infection of *S. sclerotiorum* of sunflower by half, with increase in yield by 15–25 per cent (Lukashevich, 1964b). Seed treatment with bacterial cultures, in combination with phospho-bacterin silicate is reported to control the disease giving increased yield by 13–19 per cent (Lukashevich, 1964a). Coating seeds with *Trichoderma harzianum* conidia reduces the pre and post-emergence effect of *S. sclerotiorum* in cucumber by 69 and 80 per cent respectively and in lettuce by 46 and 72 per cent, respectively. In sunflower, significant reduction (68–84 per cent) in disease incidence are obtained by incorporating the peat-bran *T. harzianum* preparation into the seedling rooting mixture (Inbar et al., 1996). Spraying of *T. harzianum* suspension at 1.8×10^6 spores/plant on lower surface of leaves and around the plants during two to three days of continuous humidity gives good control of *S. sclerotiorum* on lettuce (Avila and Gutierrez, 1992). In China, *Gliocladium roseum* 67–1 strain has ability to grow under wide temperature range and to produce mass spores. It has great potential to control soybean stem rot caused by *S. sclerotiorum* (Zhang-Yong Hua et al., 2004). In Netherlands, application of *C. minitans* spore suspension to bean, carrot, chicory and potato crops grown in rotation in soil infested with sclerotia of *S. sclerotiorum* lead to reduction of up to 90 per cent in the number of apothecia of the pathogen (Gerlagh et al., 1995a, b, 1999). A suspension of 10^6 ml^{-1} in 1,000 l ha^{-1} (10^{12} conidia ha^{-1}) of *C. minitans* sprayed on bean plants in immediately after the appearance of symptoms results in >90 per cent infection of sclerotia of *S. sclerotiorum* which prevents their carry over (Gerlagh et al., 2003).

In Italy, *Trichoderma* and *Gliocladium catenulatum* are reported to kill 96–100 per cent sclerotia of *S. sclerotiorum* (Zazzerini and Tosi, 1985). In Brazil, two isolates of *T. koningii* killed 100 per cent of the *S. sclerotiorum* sclerotia within seven days. One isolate of *T. koningii* killed 100 per cent of the sclerotia within 60 days under field conditions when soil is infested with 10^4, 10^6, or 10^8 conidia/g (Dos Santos and Dhingra, 1982). According to Wright et al. (1988), two isolates of *T. koningii* (TKI and TK2) and one of *T. viride* (TV) are the most antagonistic towards both *S. sclerotiorum* and *S. minor*.

According to Anas and Reeleder (1987, 1988a), larvae and adults of *Bradysia coprophila* act as predators of sclerotia and mycelium of *S. sclerotiorum* in the top

2 cm soil to destroy them. Colonization of senescent petals by *Alternaria alternata* and *Cladosporium cladosporioides* prevents establishment of *S. sclerotiorum* (Boland and Hunter, 1988).

Penicillium citrinum isolated from sclerotia of *S. minor* in peanut fields inhibits growth of the fungus on PDA. Citrinin, a biocide has been identified in the filtrate of *P. citrinum* as an active component against *S. minor* (Melouk et al., 1985). Field observations indicated that about 50 per cent of sclerotia of *S. minor* recovered from soil are colonized by *P. citrinum* (Akem and Melouk, 1985).

In-vitro tests revealed that vegetative growth and ascospore germination of *S. sclerotiorum* causing basal pod rot and rot of dry beans is inhibited by diffusible metabolites induced by *Bacillus cereus* strain alf-87 A. *In vivo* studies showed that the antagonistic strain alf-87 A, when sprayed on to pea plants at the pod development stage, reduces the incidence of basal pod rot from infection by airborne ascospores of *S. sclerotiorum* by 39–55 per cent. This treatment also significantly reduces the severity of basal pod rot by decreasing lesion size. Strain alf-87 A, significantly reduces the incidence of end pod rot (Huang et al., 1993). *Bacillus subtilis* metabolites inhibit mycelial growth of *S. sclerotiorum* on bean and wheat seeds and also soil-borne fungus (Lazzaretti et al., 1994). In the field trials conducted for *Sclerotinia* stem rot control of canola, *Pseudomonas chlororaphies* (PA-23), *Pseudomonas* spp # 41, *Bacillus amylolique faciens* (BG 6) and *Staphylococcus* spp (E-16) significantly reduced disease (Zhang, 2004). Reduction in biomass of *S. sclerotiorum* has been observed on addition of various levels of *Pseudomonas maltophila* cells 10^4–10^8 cells (Kohli et al., 2006).

Fusarium oxysporum (S6) is a good candidate for the biocontrol of *S. sclerotiorum* in soybean (Rodriguez et al., 2006). *T. sclerotivorum* applied at the rate of 20 spores/cm^2 soil has the potential to become a successful biocontrol agent of *Sclerotinia* stem rot of soybean (Rio et al., 1998).

19.10 Mechanism of Biological Control

The mechanisms of parasitism appear to differ significantly among several mycoparasite of *Sclerotinia* spp. Sclerotia of *S. sclerotiorum* and *S. minor* dried for short periods then remoistened in soil, lack nutrients are rapidly colonized by other microorganisms and rot within two to three weeks (Smith, 1972). A mechanism of killing of *S. sclerotiorum* by some of the hyperparasitic fungi has been worked out. *Coniothyrium minitans* kills both sclerotia and vegetative hyphae as a result of direct penetration of hyphae of *S. sclerotiorum* and causes collapse of the protoplasm (Hoes, 1977; Hoes and Huang, 1975; Huang and Hoes, 1976). *C. minitans* A69 protects lettuce plants from infection during the first four weeks after suppressing mycelial growth of the pathogen through localized antibiosis and hyphal parasitism. *C. minitans* also makes contact with and parasitize sclerotia in the vicinity of the developing plant and thereby reduce the inoculum which may contribute to mid late season infection (Stewart et al., 2000). But *Gliocladium catenulatum*

kills the *S. sclerotiorum* by making only the hyphae contact without penetration. Jones et al. (1974) reported that β-(1–3)-glucanase and chitinase are the key enzymes involved in the destruction of cell walls of *S. sclerotiorum* by *C. minitans* and *Trichoderma viride*. Ghaffar (1972) speculated that melanolytic enzymes played a role in permitting entry though the sclerotial rind. Crude enzyme extracts of *C. minitans* lyse the pseudparenchymatous tissue of inoculated sclerotia (Jones and Watson, 1969). Purified glucanases have been separated and shown to degrade 'sclerotan' a major cell wall component of the sclerotia (Bacon et al., 1972; Jones et al., 1974). *Microsphaeropsis centaureae* possibly lyses the sclerotia tissue in a similar manner (Watson and Miltimore, 1975). Some kind of enzymatic mechanism may be involved in the breakdown of the pseudoparenchymatous tissues of sclerotia by *T. viride* (Dos Santos and Dhingra, 1982; Jones et al., 1974). According to Lee and Wu (1979), *T. harzianum* disintegrate the cell walls of *S. sclerotiorum* and causes the hyphae of *S. sclerotiorum* to become swollen as well as to release cytoplasm. *Penicillium* spp., *Aspergillus* spp. and *Bacillus* spp. produce some antibiotic substance and inhibit the growth of *S. sclerotiorum*.

Gliocladium virens forms appressorium-like structures on the host fungus and achieves its infection by active penetration (Plates 19.10.1, 19.10.2-Figs. 5–12). *G. virens* parasitize sclerotia internally which are incapable of either myceliogenic or ascocarpic germination (Tu, 1980). It is active over a broad range of soil moisture levels and over the entire agricultural soil pH range (pH 5–8). Although active parasitism takes place in soil over the range of 15–35°C, but parasitism at 15°C is greatly reduced compared with that at higher temperature (Phillips, 1986a).

On bean leaves fungi like *Alternaria alternata* and *Cladosporium cladosporioides* compete for nutrients with *S. sclerotiorum* and act as antagonists rather than parasites or antibiosis (Boland and Hunter, 1988a). According to Huang and Kokko (1993) sclerotia of *S. sclerotiorum* causing bean mould disease near Lethbridge, Canada can be infected and killed up to 54 per cent if incubated for four weeks after inoculation with *Trichothecium roseum* on moist sand. Transmission electron microscopic studies of infected sclerotia revealed that hyphae of *T. roseum* enter the rind tissue by penetrating the melanized cell walls or via junctions between cells. Lysis of host cell walls occurs at penetration sites. Hyphae of *T. roseum* ramify in cortical and medullary tissues, destroying the sclerotium. In sclerotia with light infections of *T. roseum*, numerous cortical and/or medullary cells show cytoplasmic granulation and vacuolization without direct association with the mycoparasitic hyphae (Plates 19.10.3–19.10.7-Figs. 11–13).

Sclerotia inoculated with spores of *Talaromyces flavus* are colonized by the hyperparasite after 3, 7 or 12 days and often the tissue becomes soft and decayed. The hyphae of *T. flavus* penetrate the wall of the rind cells and grow inter and intracellularly within the sclerotia (McLaren et al., 1987, 1989).

Epicoccum purpurascens inhibits germ-tube elongation of ascospores on leaf discs of lettuce and reduce infection of lettuce plants by *S. sclerotiorum* in growth chamber tests (Mercier and Reeleder, 1987). This inhibition is thought to be due to production of antifungal compounds by *E. purpurascens*. Application of *E. purpurascens* conidia to bean plants effectively controls white mould in greenhouse

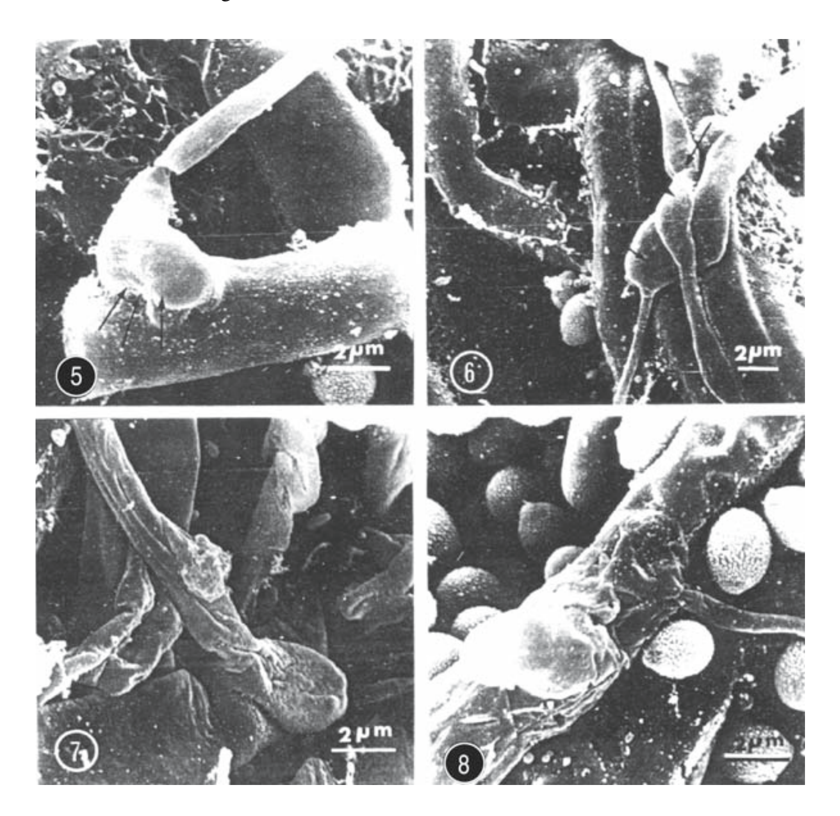


Plate 19.10.1 Scanning electron micrographs of the parasitization of *Sclerotinia sclerotiorum* by *Gliocladium virens*. (Figs. 5, 6) Various shapes and size of appressoria (arrow) formed by *G. virens* on the mycelia of *S. sclerotiorum*; (Fig. 7) shrinkage of appressoria after penetration into the host hyphae; (Fig. 8) shrinkage of host hyphae due to intercellular parasitism of the mycoparasite (Adapted from the publication of Tu, 1980. With permission)

and field trials (Zhou and Reeleder, 1989). Sterile culture filtrates of *E. purpurascens* decreases severity of white mould of bean and increases pod yield when applied prior to inoculation of bean with *S. sclerotiorum*. Extracts of these culture filtrates inhibit ascospore germination and mycelial growth of *S. sclerotiorum* (Zhou et al., 1991).

Damage to sclerotia of *S. sclerotiorum* caused by larvae of *Bradysia coprophila* is highest in soils which have organic matter content >75 per cent nitrate concentrations of more than 100 μg g^{-1} of soil and pH values between 4.4 and 5.2 (Anas and Reeleder, 1987). When sclerotia are placed in soils infested with *T. viride*, it is found that sclerotia that had been grazed by larvae are more susceptible to colonization by *T. viride* than undamaged sclerotia (Anas and Reeleder, 1988b). While feeding on sclerotia of *S. sclerotiorum*, larvae of *B. coprophila* deposit salivary secretions on the sclerotial surface. Sclerotia damaged by the feeding activities of the larvae have a decreased ability to germinate (Anas et al., 1989). According to Garacia-Garza et al. (1997), fewer sclerotia of *S. sclerotiorum* survive when

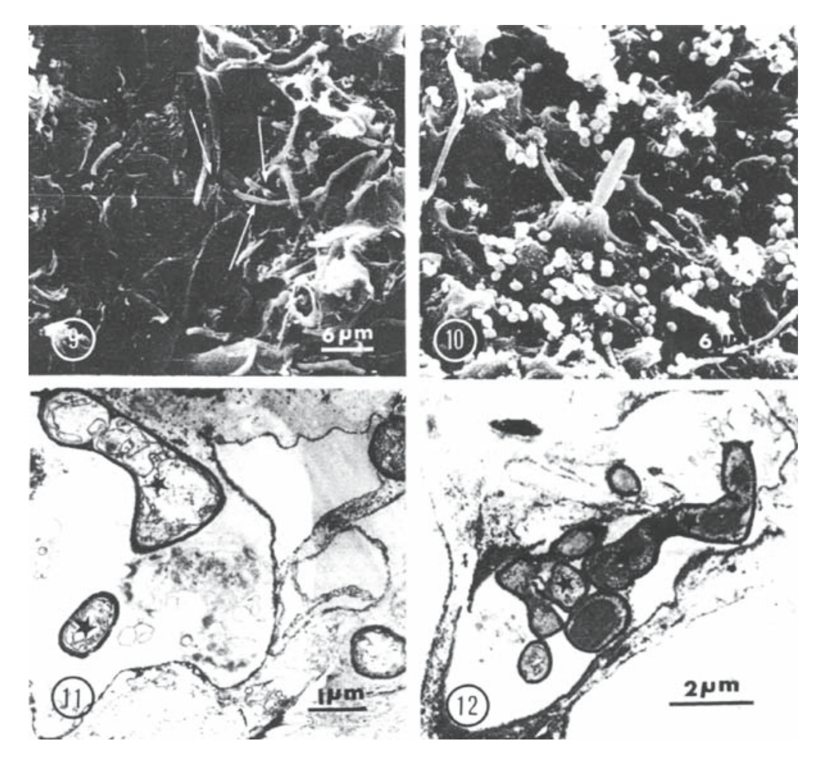


Plate 19.10.2 Scanning and transmission electron micrographs of extra and intracellular parasitization of *Sclerotinia sclerotiorum* by *Gliocladium virens*. (Fig. 9A) Scanning view of broken sclerotium showing many extracellular and intracellular hyphae (arrow); (Fig. 10) spores of *G. virens* were found exclusively on the surface of the parasitized sclerotia; (Fig. 11) micrograph of thin section showing both extracellular [between cell walls of sclerotial cells (arrow)]; (Fig. 12) extensive intracellular invasion of sclerotia by mycoparasitic hyphae (asterisks) as observed in micrograhs of thin section (Adapted from the publication of Tu, 1980. With permission)

T. hamatum isolate TMCS3 is combined with fungus gnats (*Bradysia coprophila*) in the soil treatment.

Coniothyrium minitans and certain isolates of *Trichoderma* species appear to be firmly established as mycoparasite of sclerotia of *Sclerotinia* spp. and in some soils may be responsible for natural destruction of sclerotia (Adams and Ayers, 1979). The other mycoparasite named *Sporidesmium sclerotivorum* (Uecker et al., 1978, 1980) and *Teratosperma oligocladum* (Ayers and Adams, 1981a; Parfitt et al., 1983) if introduced in to natural soil brings 95 per cent reduction of the inoculum density of *S. minor* within ten weeks at 20°C. An unusual property of *S. sclerotivorum* is its ability to grow through soil from one sclerotium to another, producing many new conidia throughout the soil mass (Ayers and Adams, 1979b). The mycoparasite *S. sclerotivorum* has been detected in soils of several states of USA. The mycoparasite applied at 100 spores per gram of soil is responsible for a decline in the survival of sclerotia (Adams and Ayers, 1981). In a recent field trial, *S. sclero-*



Plate 19.10.3 Transmission of electron micrographs showing hyphae of *Trichoderma roseum* (TR) penetrating (Fig. 1 arrow), the melanized rind cell walls of a sclerotium of *Sclerotinia sclerotiorum*; (Figs. 2, 3) are serial sections, 13 sections apart taken from the area outlined in Fig. 1. Note lysis of the melanized cell walls (CWS) at the site of penetrating by hypha of *T. roseum* (TR). CWT cell wall of *T. roseum*. CWS Cell wall of *S. sclerotiorum* (Adapted from the publication of Huang and Kokko, 1993. With permission)

tivorum provided 63–83 per cent control of *Sclerotinia* disease of lettuce over a three year period (Adams and Ayers, 1982). The optimum conditions for infection and decay of sclerotia in soil are 20–25°C, pH 5.5–7.5 and soil water potentials of −8 bars and higher. Infection and decay of sclerotia occurs after a sand sclerotia culture of *S. sclerotivorum* is added to soils at rates as low as 2×10^3 spores per 100 g of soil (Adams and Ayers, 1980). *S. sclerotivorum* can survive in moist and air-dried soils stored at room temperature for 15 months. Cultivation of *S. sclerotivorum* parasitically on living sclerotia proceeds optimally in moist, fine quartz sand amended with 1–2 per cent (W/W) sclerotia and 0.07 per cent (W/W) $CaCO_3$,

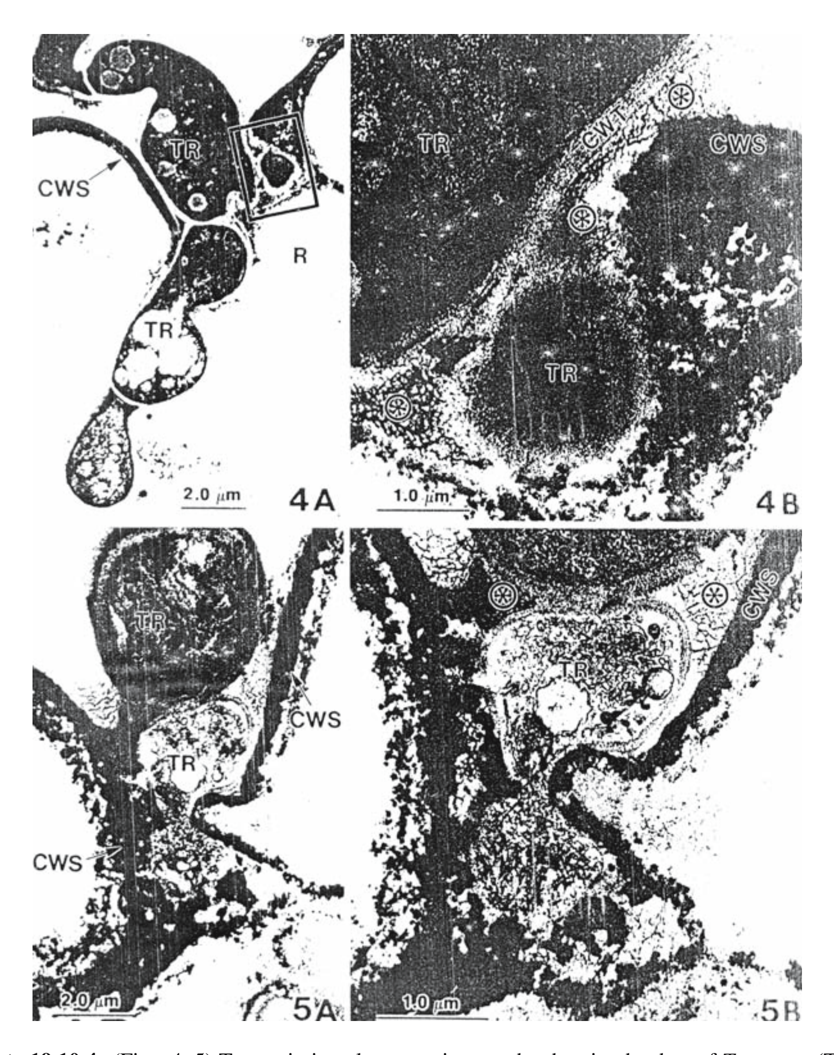


Plate 19.10.4 (Figs. 4, 5) Transmission electron micrographs showing hyphae of *T. roseum* (TR) penetrating intercellular junctions between rind cells (R) of *S. sclerotiorum.* (Figs. 4A–5A) Lower magnification showing penetration sites. (Figs. 4B–5B) Higher magnification of Fig. 4A (area outlined) and Fig. 5A, showing array of fibrous net like structures (*) connected to cell walls of *T. roseum* and etching of the melanized cell walls (CWS). CWT-cell wall of *T. roseum*. CWS-cell wall of *S. sclerotiorum* (Adapted from the publication of Huang and Kokko, 1993. With permission)

at 25°C. Infection of sclerotia in sand reaches 100 per cent by five weeks (Ayers and Adams, 1979a). Adams and Fravel (1990) developed an economical biological control of *Sclerotinia* lettuce drop by *Sporidesmium sclerotivorum* under field conditions. However, *S. sclerotivorum* is difficult to grow *in vitro* limiting production of large scale quantities of the inoculum (del Rio et al., 2002).



Plate 19.10.5 A transmission electron micrograph showing *T. roseum* (TR) hyphae ramifying throughout the cortical (C) and medullary (M) tissues of sclerotium of *Sclerotinia sclerotiorum*. The cell walls of the rind layer (R) remain intact but the cell walls of the cortical and medullary tissues are disintegrated (Adapted from the publication of Huang and Kokko, 1993. With permission)

The *Phragmosporum* macroconidia of *S. sclerotivorum* and *Teratosperma oligocladum* germinate and infect adjacent sclerotia of susceptible species within five days *in vitro* and in soil (Ayers and Adams, 1979b, 1981). From infected sclerotia, the mycoparasite send out thread like hyphae that produce many new conidia throughout the soil spaces. The conidia persist in soil for extended periods. Living sclerotia are more frequently and abundantly colonized than autoclaved sclerotia by *S. sclerotivorum* (Ayers and Adams, 1979a) and neither mycoparasite appear to invade hyphae of *Sclerotinia* spp. (Ayers and Adams, 1979a, 1981). Infected sclerotia recovered from soil typically become soft and mushy and in the later stages, readily disintegrate when touched. Macroconidia of both the mycoparasite when applied to sclerotia of *S. minor* or *S. sclerotiorum* germinate within three to five days on the surface of the sclerotia. The germ tube penetrates the rind and proliferates beneath the surface of the sclerotia. In sectioned sclerotia, the germ tubes of the mycoparasite can be seen penetrating between the cells of the rind and cortex without aid of specialized penetration structures (Plate 19.10.8-Figs. 1–15) (Adams and Ayers, 1983). However, later, Bullock et al. (1986) through light and transmission electron microscopic observations revealed large numbers of hyphae in the extra cellular matrix of the sclerotia and intracellular structures in the cortical and medullary hyphae. These intracellular structures are interpreted as haustoria of the mycoparasite (Plate 19.10.9-Figs. 1–13). Frequently hyphal strands on the sclerotial surface branch and each branch appear to infect the sclerotium, resulting in multiple infections. Once within the medullary region of the sclerotium, the infection hyphae branch and grow out intercellularly. The hyphae are convoluted

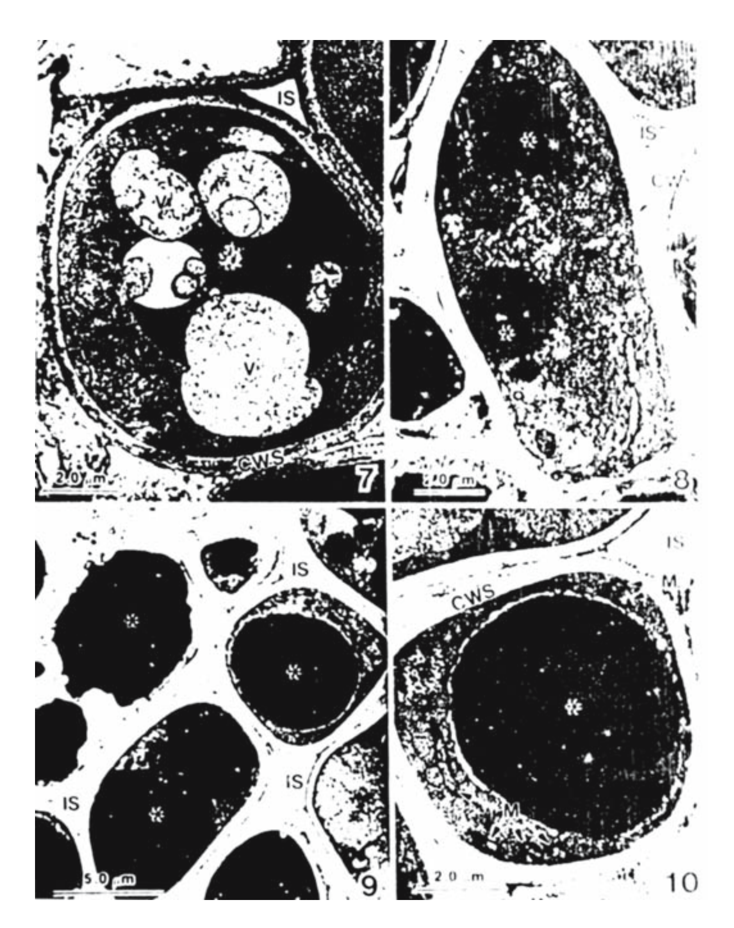


Plate 19.10.6 (Figs. 7–10) Transmission electron micrographs showing cytoplasmic changes of cortical cells of a sclerotium of *S. sclerotiorum* infected by *T. roseum*. Note cortical cells are free of hyphae of *T. roseum*, yet vacuoles (V) are present in the cytoplasm (Fig. 7); and there are lightly and darkly stained granular inclusions (Figs. 8, 9, 10). CWS cell wall of *S. sclerotiorum*; (M) mitochondria; (IS) intercellular space (Adapted from the publication of Huang and Kokko, 1993. With permission)

and assume the shape of the intercellular spaces. After proliferating within the medulla, the mycoparasite grow to the surface of the sclerotium where it sporulates abundantly. Sclerotial cells are not invaded by either mycoparasite. Live sclerotia are much more extensively invaded than autoclaved sclerotia. Glucanase activity that hydrolyze and β-glucans are detected in non-infected sclerotia of *S. minor* and *S. sclerotivorum* and glucans are extracted from host sclerotia. Specific β-glucanase activity increases by infection of sclerotia by *S. sclerotivorum. S. sclerotivorum* does not grow in a medium with glucan of *Sclerotinia* as sole carbon source but does so when the glucan is previously incubated with glucanase extracted from host sclerotia. The mycoparasite utilize glucose and possibly other monosaccharides released from the extra cellular matrix of the medulla by physiological interactions of the enzymatic systems of their hosts.



Plate 19.10.7 (Figs. 11–13) Transmission electron micrographs showing cytoplasmic changes in medullary cells of a sclerotium of *S. sclerotiorum* infected by *T. roseum*. Note medullary cells are free of hyphae of *T. roseum*, yet vacuoles (V) are formed in the cytoplasm (Fig. 13A, B) and there is cytoplasmic granulation (*) (Figs. 11–13). Note also that parts of the vacuolized (Fig. 13A) and (or) granulated (Figs. 11–13). Cytoplasm remains relatively intact. (M) Mitochondria; (IS) intercellular space; (W) Woronin body, (Fl) fibrous layer, (CWS) cell wall of *S. sclerotiorum* (Adapted from the publication of Huang and Kokko, 1993. With permission)

Infection of hyphae of *S. sclerotiorum* by the hyperparasite *Coniothyrium minitans* has been reported by several workers (Huang and Hoes, 1976; Trutmann et al., 1982; Tu, 1984), but these studies did not agree completely on the mode of hyperparasitism. Using light microscopy, Huang and Hoes (1976) observed that hyphal tips of *C. minitans* invaded hyphae of *S. sclerotiorum* by direct penetration without forming any special structure. Trutmann et al. (1982) also observed direct hyphal penetration but penetration of host hyphae is achieved only by tips of side branches of the hyperparasite, not by tips of the main hyphae. Using scanning electron microscopy (SEM), Tu (1984) observed that hyphae of *C. minitans* produces appressoria when come in contact with the undamaged hyphae of *S. sclerotiorum*

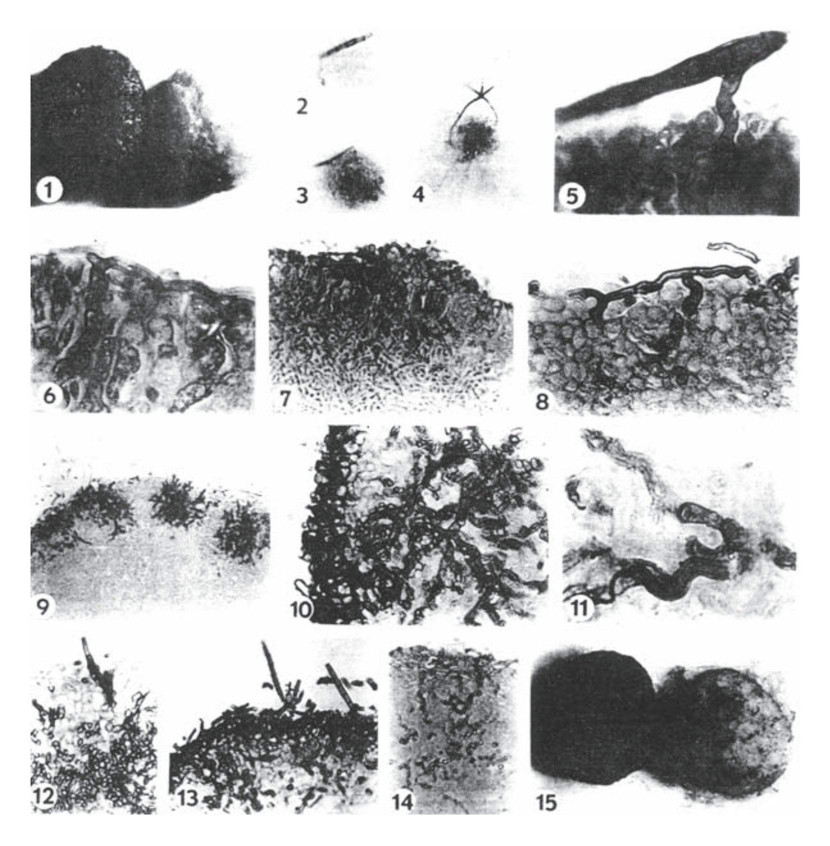


Plate 19.10.8 (Figs. 1–15) Photomicrographs of invasion of sclerotia of *Sclerotinia* spp. by *S. sclerotivorum* and *Teratosperma oligocladium*. (Fig. 1) Sclerotia of *S. sclerotivorum* isolate Ss-3 (left) and tan isolate Ss-60 (right). Six days after inoculation with *T. oligocladium*. (Fig. 2) Germinating macroconidia of *S. sclerotivorum* on surface of isolate Ss 60 (three days). (Fig. 3) Penetrating of surface of isolate Ss-60 by germinated macroconidium of *S. sclerotivorum* (five days). (Fig. 4) Germination and penetration of isolate Ss-60 by macroconidium of *T. oligocladium* (ten days). (Figs. 5, 6) Penetrating of rind and cortex of sectioned sclerotium of Ss-60 by germinated conidium of *T. oligocladium* (ten days). (Fig. 7) Early stages of infection of isolate Ss-60 by *T. oligocladium* with raised surface following penetration of cortex (ten days). (Fig. 8) Hyphae of *T. oligocladium* on the surface of surface of sectioned sclerotiorum of isolate Ss-60 with multiple points of infection (21 days). (Fig. 9) Sectioned sclerotiorum of isolate Ss-60 with multiple areas of infection by *S. sclerotivorum* (21 days). (Fig. 10) Extensive developments of mycelium of *S. sclerotivorum* within the medulla of *S. minor* isolate Ss-13. (Fig. 11) Mycelium of *S. sclerotivorum* within the medulla of *S. sclerotivorum* within sclerotium of Ss-60. Note that mycelium is restricted to the extra cellular matrix between the medullary cells (21 days). (Fig. 12) Abundant development of *S. sclerotivorum* within sclerotium of Ss-60 and exit hyphae of the mycoparasite (29 days). (Fig. 13) Macroconidium of *S. sclerotivorum* sporulating on a sclerotium of *S. minor* naturally infected in the field. (Fig. 14) Sparse colonization of an autoclaved sclerotium of isolate Ss-60 by *S. sclerotivorum*. Compare with heavily invaded live sclerotium in Fig. 12 inoculated at the same time (29 days). (Fig. 15) Surface development of *T. oligocladium* on invaded sclerotium of isolate Ss-3 and Ss-60. Sclerotia are the same as in Fig. 1 (16 days) (Adapted from the publication of Adams and Ayers, 1983. With permission)

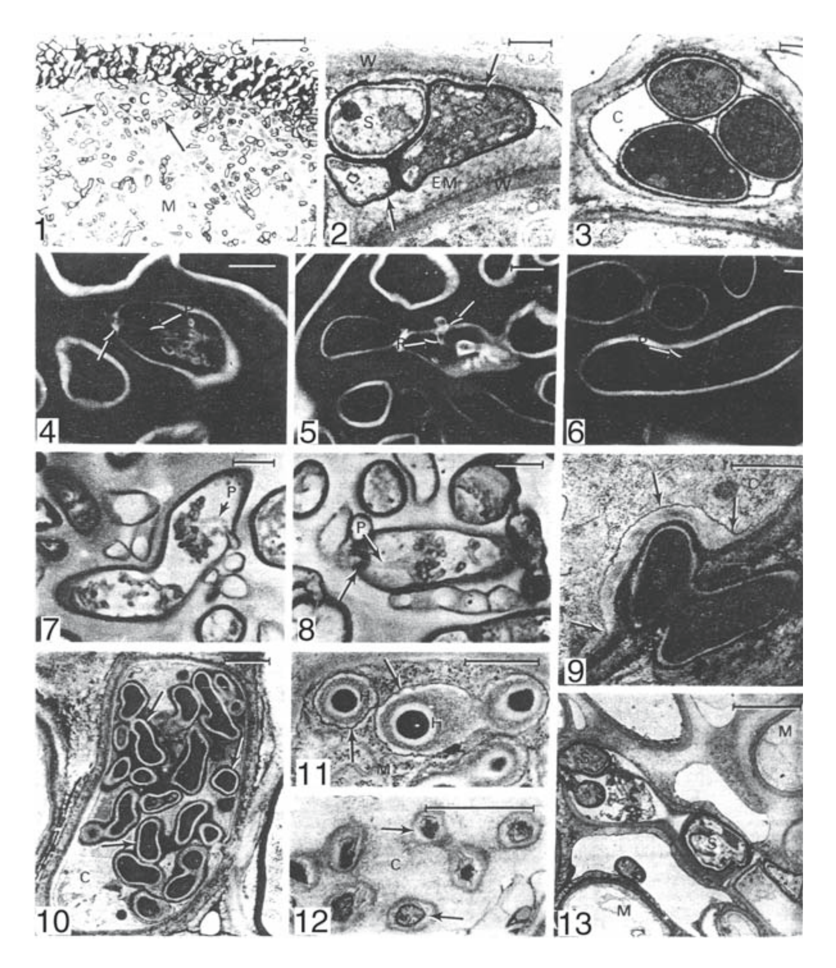


Plate 19.10.9 Light (LM) and transmission electron micrographs (TEM) of *Sporidesmium sclerotivorum* in sclerotial tissue of *S. minor*. (Fig. 1) LM; showing hyphae of *S. sclerotivorum* (arrow) in the cortex © and medulla (M), 20 days after inoculation. Toluidine blue O stain. (Fig. 2) TEM; showing hyphae of *S. sclerotivorum* (S) growing in the extra cellular matrix (EM) of the medulla. Their walls are thinner and more electron opaque (arrow) than those of the medullary hyphae (W). 30 days (Fig. 3) TEM showing thin walled hyphae of *S. sclerotivorum* (S) in an empty cortical cell © 15 days (Figs. 4–8) Light micrographs of haustoria of *S. sclerotivorum* in medullary cells. The long penetrating hyphae (P) are branched at their distal ends. There is a deposit (arrow) on the sclerotial cell walls at the point of penetration. (Figs. 4–6) 15 days calcoflour white M2R stain. (Figs. 7, 8) 20 days, PAS stain. (Fig. 9) TEM of hypha of *S. sclerotivorum* (S) that penetrating the walls of cortical cell (C) showing the many branches of a hautorium (H) in section. An electron translucent region surrounds each branch (arrow). 30 days (Fig. 10) TEM of cortical cell (C) showing the many branching of a hautorium (H) in section. An electron translucent region surrounds each branch (arrow). 30 days. (Fig. 11) TEM showing details of haustoria branches (H) with dense cytoplasm in a medullary cell (M). The surrounding sheath is delimited by a unit membrane (arrow) 20 days. (Fig. 12) TEM of degenerate haustorial branches (arrow) in a cortical cell (C). The cytoplasm of the branches is disrupted 40 days. (Fig. 13) TEM showing hyphae of *S. sclerotivorum* (S) in the outer medulla (M). The cytoplasm of both *S. sclerotivorum* and sclerotial cells has degenerated 40 days (Adapted from the publication of Bullock et al., 1986 with permission).

in dual culture on PDA and that the appressoria are about twice the diameter of the hyphae of *C. minitans*. The parasitized hyphae gradually shrink and collapse and hyphae of the mycoparasite are found inside the host hyphae. The mycoparasite hyphae grow inter- and intracellularly within the sclerotia of *S. sclerotiorum*. In the later stages of parasitism, hyphae of the mycoparasite proliferate extensively within the sclerotia and form pycnidia near the sclerotial surface. At this stage, the sclerotia become flattened, soft and disintegrated. Sclerotia parasitized by *C. minitans* fail to germinate either myceliogenically or carpogenically. However, study by Huang and Kokko (1987) using scanning electron microscopy (SEM) confirms the previous reports that hyphal tips of *C. minitans* invade the host hyphae by direct penetration without developing appressoria and that indentation of the host cell wall at the point of penetration is often evident. There is no functional distinction between a main branch and a side branch hypha of the hyperparasites and tips of either type of hyphae are capable of invading host hyphae by direct penetration (Plate 19.10.10–19.10.13-Figs. 1–12). Water soluble extracts of bean leaves or flower stimulates spore germination and hyphal growth of *C. minitans*. Thus colonization of senescent tissues such as bean flowers by *C. minitans* is an effective mechanism for suppression of white mould of bean caused by *S. sclerotiorum* (Bremer et al., 2000). *Pseudomonas maltophila* has been found to produce chitinase which is responsible for the lysis of mycelial biomass of *S. sclerotiorum*. This bacterium shows good growth on chitin and cell wall preparation of the fungus (Kohli et al., 2006).

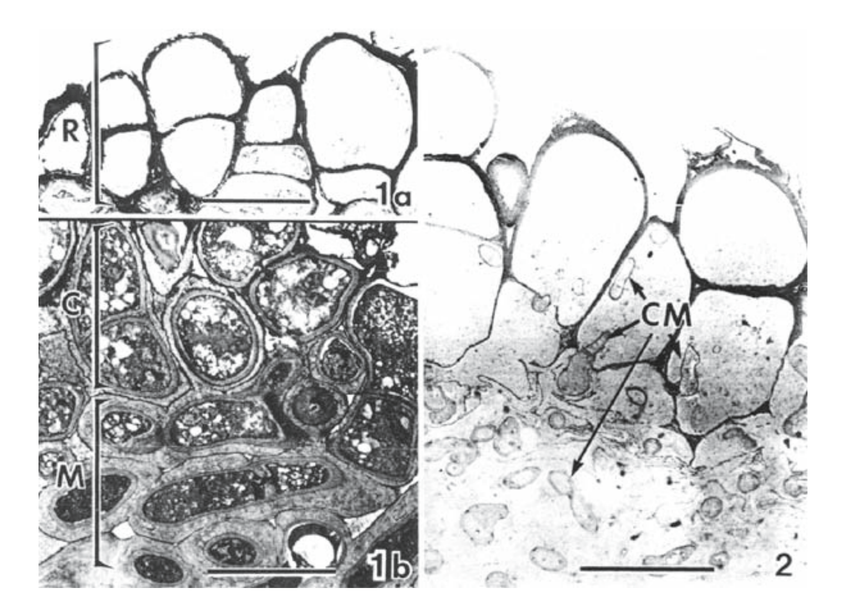


Plate 19.10.10 Cross section of healthy sclerotium of *Sclerotinia sclerotiorum* showing three distinctive layers of tissue; rind (R) (Fig. 1a); Cortex (C) and medulla (M), (Fig. 1b). (Fig. 2) Cross section of sclerotium infected with *C. minitans* showing complete destruction and disintegration of cortical and medullary tissues of mycelia of hyperparasite (CM). The rind is infected but remains intact (Adapted from the publication of Huang and Kokko, 1987. With permission)

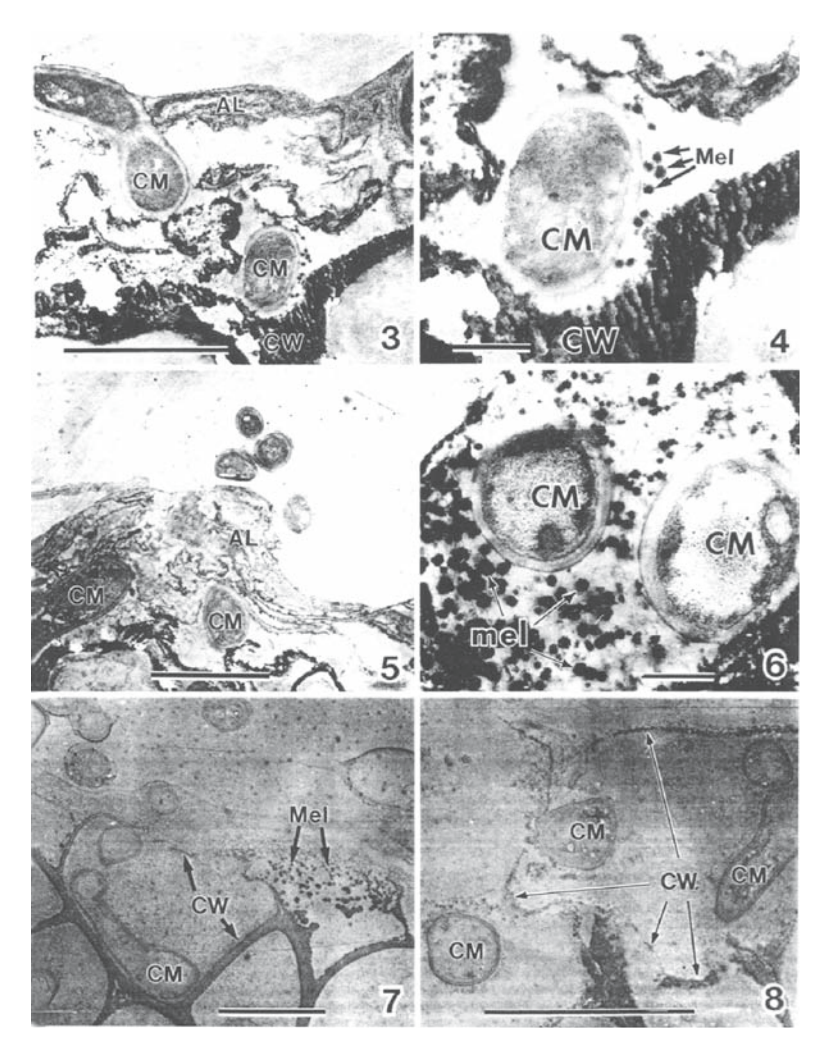


Plate 19.10.11 Invasion of rind by *C. minitans*. Note the hyphae of *C. minitans* (CM) in the amorphous layer (AL) (Figs. 3, 5) and the thick melanized wall (CW). Note the sign of wall etching by the hyperparasite (CM) (Fig. 4) and the loose melanin particles (mel) near the affected cell wall (Figs. 4, 6). (Figs. 7, 8) Destruction of outer rind cells by *C. minitans* (Adapted from the publication of Huang and Kokko, 1987. With permission)

The interactions of *S. sclerotiorum* and seven biological control agents (BCAs) have been examined in controlled environments to determine the influence of RH (90–100 per cent) and air temperature (20–28°C) on biological control of white mould of bean (Hannusch and Boland, 1996). Changes of 4°C or 5 per cent RH are associated with variability in disease suppression that range from less than or equal to 25–100 per cent. *Epicoccum nigrum* is comparatively independent of environment and suppresses disease by 100 per cent in all environments. Suppression of

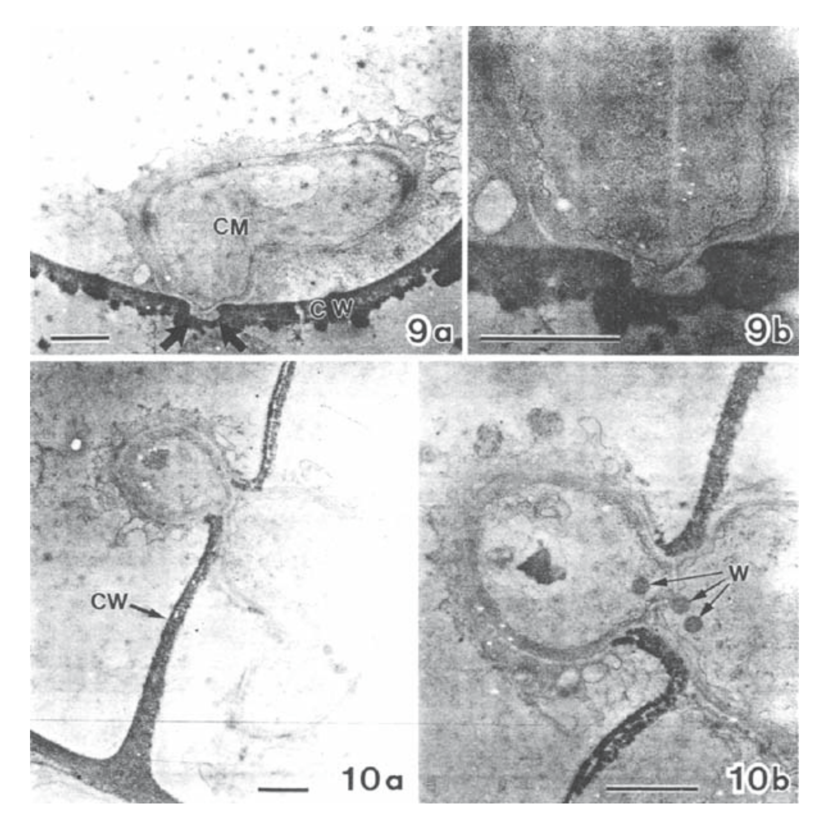


Plate 19.10.12 (Fig. 9) Early stage of penetration of the melanized wall of a rind cell (CW) by *C. minitans* (CM) showing a small, well defined gap (Fig. 9a) (arrows) created by the narrow penetration peg. Invagination of the host cell wall at the penetration site is not evident. (Fig. 10) Invagination of the melanized wall (CW) at the penetration site is evident when penetration is complete. Note the narrow hyphal neck of the hyperparasite with Woronin bodies (W) in it (Fig. 10b) (Adapted from the publication of Huang and Kokko, 1987. With permission)

disease by many of the BCAs is most effective under environmental conditions that are least conducive for disease.

To assess the ability of fungi antagonistic to *S. sclerotiorum* to inhibit the formation of sclerotia and to grow through plant tissue from cut surfaces, a plant-tissue based system has been developed by Whipps (1987) using celery, lettuce and tomato segments. *Gliocladium roseum* and three isolates of *Trichoderma harzianum* have significant ability to grow through plant tissue and significantly decrease the production of sclerotia when plant tissues are treated before or along with *S. sclerotiorum*. In dual culture *T. harzianum* hyphae grow towards and coil around the *S. sclerotiorum* hyphae. Dense coils of hyphae of *T. harzianum* and partial degradation of the *Sclerotinia* cell wall are observed in later stages of the parasitism. In sterile soil, conidia of *T. harzianum* germinate and the developing

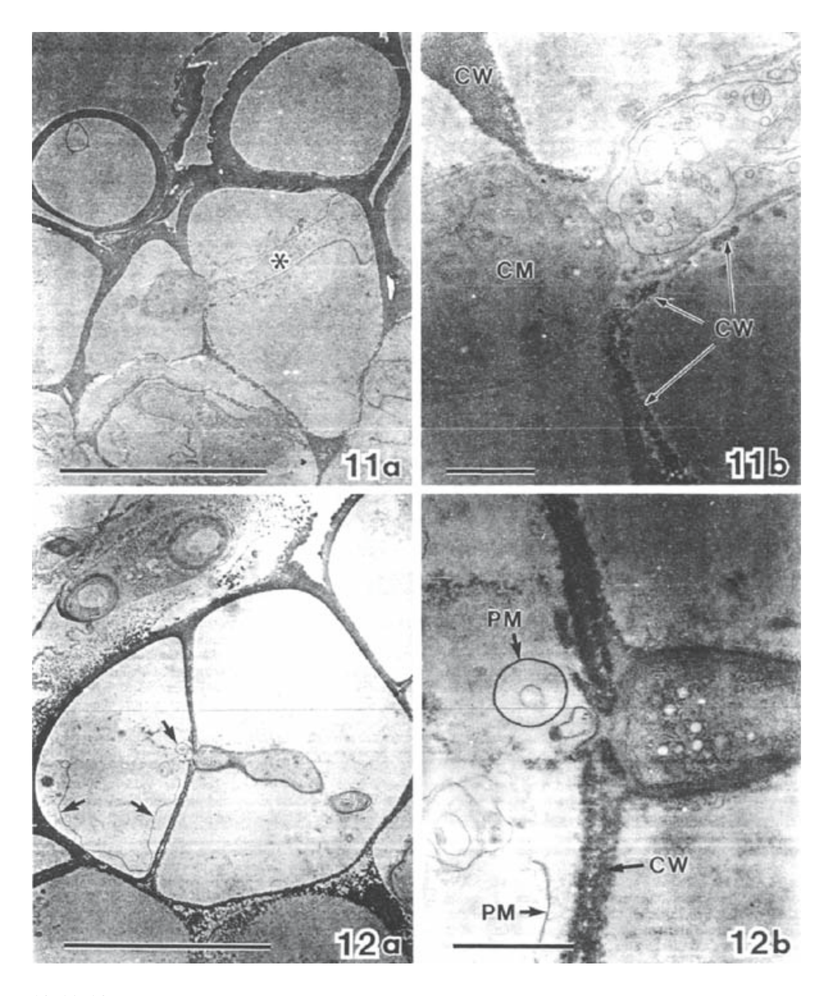


Plate 19.10.13 (Figs. 11, –12) Lysis and death of young cells of *C. minitans.* (Figs. 11a–12a) in infected rind tissue. The wall of the dead cell is either partially disintegrated (Fig. 11b) or completely disintegration with its membranous structure (PM) in the host cell (Fig. 12) (Adapted from the publication of Huang and Kokko, 1987. With permission)

mycelium makes contact with that of *S. sclerotiorum* forming short branches and appressorium-like bodies which aid in holding and penetrating the host cell wall. Hyphal mycoparasitism rather than sclerotial parasitism is suggested to be the mechanism by which *T. harzianum* controls *S. sclerotiorum* (Inbar et al., 1996).

Examination of sclerotia by transmission E.M. at 3, 7 and 12 days after inoculation revealed that hyphae of *Talaromyces flavus* penetrate the rind cell walls of *S. sclerotiorum* directly. Etching of the cell wall at the penetration site is evident. This suggests that wall lysing enzymes may be involved in the process of infection. Hyphae of *T. flavus* grow both inter-cellularly and intra-cellularly throughout the

rind, cortical and medullary tissues. Ramification of the hyperparasite in the sclerotium results in destruction and collapse of sclerotial tissues (McLaren et al., 1989).

Parasitism by *T. koningii* of sclerotia of *S. sclerotiorum* is favoured by the presence of exogenous nutrients and is maximum at temperatures between 20–30°C. At 20°C, *T. koningiii* requires two weeks to infect 50 per cent of sclerotia, there is an inverse relationship between infection and sclerotial viability. *T. koningii* is able to penetrate dry bean stems and to infect a proportion of sclerotia lodged within the pith. It has no effect on emergence and development of *Phaseolus vulgaris*. Mycoparasitism is necrotrophic and involves destruction of hyphae of *S. sclerotiorum* upon contact. Coiling of *T. koningii* around its host hyphae is common. Extensive growth of *T. koningii* within hyphae of *S. sclerotiorum* is achieved without production of specialized infection structures. At later stages of parasitism, *T. koningii* sporulate on remnants of hyphae of *S. sclerotiorum*. Application of *T. koningii* as a pre-sowing treatment for *S. sclerotiorum* in spring and summer results in significant reductions in the viability of sclerotia and significant increase in the number of sclerotia infected with *T. koningii*. Its application as a post harvest soil treatment in winter for sclerotia protected in stems is not effective. *T. koningii* has potential as a biocontrol agent for *S. sclerotiorum* when applied in warmer months or in warmer climates (Trutmann and Keane, 1990).

According to Liu (1989), *Gliocladium deliquescens* and *Penicillium vermiculatum* hyphae also coil around and cause shrinkage of *S. sclerotiorum* hyphae. Bioagents can also invade and decay the sclerotia. This may be due to production of β-(1–3) glucanase and chitinase which lyse the host cell wall and cause sclerotial decay. The development of resistance in *Sclerotinia* against the biological agent *Bacillus subtilis* CL-27 has been observed by Li and Leifert (1994). According to Adams (1989), *S. sclerotivorum* and *S. oligocladum* are aggressive mycoparasites whereas other antagonists are passive mycoparasites.

19.10.1 Use of **Sporidesmium sclerotivorum** *as Biological Control*

Sporidesmium sclerotivoruim is a very slow growing biotroph attacking only sclerotia of fungi in the Sclerotiniaceae, yet, *S. sclerotivorum* applied at rates as low as 0.2 kg/ha successfully controls lettuce drop caused by *Sclerotinia minor* (Adams and Fravel, 1990; Ayers and Adams, 1981a; Fravel et al., 1992; Adams and Fravel, 1993). Control is based on a thorough ecological understanding of the organisms involved. Control is successful because the natural attributes of the crop, cropping system, pathogen and biocontrol agent are all exploited to achieve control. Studying *S. sclerotivorum* provides insight about how, when and where biocontrol by a mycoparasite is likely to work, as well as how to screen for new mycoparasites.

Sporidesmium sclerotivorum is a soil inhabiting, dematiacious hyphomycete that produces macroconidia, microconidia and microsclerotia (Uecker et al., 1978). It is one of the few dematiaceous hyphomceytes that produces holoblastic, phragmoseptate

conidia which are blown out ends of conidiophores. The macroconidia are unusually large (60–92 × 6–8 µm), which facilitates recovery and identification of this antagonist. *Sporidesmium sclerotivorum* is widely distributed throughout temperate areas of the world (Adams and Ayers, 1981, 1985; Chaban et al., 1993; Litkei, 1988). *Sporidesmium sclerotivorum* has been found in both organic and mineral soils with a range of pH from 5.5 to 6.5 (Adams and Ayers, 1985).

In nature, *S. sclerotivorum* behaves as an obligate parasite on sclerotia of species of *Sclerotinia*. In addition to *S. sclerotivorum*, number of other fungi and bacteria (Table 19.9.1) has been reported as antagonists of *Sclerotinia* spp. *Teralosperma oligocladium* a BCA fungus is morphologically similar to *S. sclerotivorum* and also parasitizes sclerotia of *Sclerotinia* spp. (Ayers and Adams, 1981b). *Teratosperma oligocladium* is distinguished from *S. sclerotivorum* by its pentaradiate conidia (Uecker et al., 1980). Macroconidia of *S. sclerotivorum* germinate near or on host sclerotia in response to the presence of the host. Glucose (Ayers et al., 1981) and an unidentified compound (Mischke et al., 1995) stimulate germination of macroconidia of *S. sclerotivorum*. The number of macroconidia which germinate is directly related to the number of host sclerotiaper gram soil (Ayers and Adams, 1979a, b). Often, multiple germ tubes arise from a macroconidium resulting in multiple penetrations of a sclerotium (Adams and Ayers, 1983).

Table 19.9.1 Antagonists of *Sclerotinia*

Antagonist	References
Acinetobacter sp.	Oedjijono and Dragar, 1993
Acremonium implicatum	Singh, 1991b
Acrostalgmus spp.	Makkonen and Pohjakallio, 1960
Alternaria alternata	Boland and Hunter, 1988
Aphelenchoides sp.	Sanudo, 1975
Aspergillus spp.	Lee and Wu, 1979
A. flavus	Makkonen and Pohjakallio, 1960
A. fumigatus	Singh, 1991b
A. niger	Singh, 1991b
A. terrus	Gupta and Aggarwal, 1988
Azotobacter chroccoccum	Suneja et al., 1994
Bacillus spp.	Lee and Wu, 1979
B. amyoliquefaciens	Fernando et al., 2004
B. cereus	Huang et al., 1993
B. macerans	Nelson et al., 2001
B. polymyxa	Nelson et al., 2001; Yuen et al., 1991
B. subtilis	Lazzaretti et al., 1994
Bradysia spp.	Anas and Reeleder, 1988a
B. coprophila	Anas and Reeleder, 1988b; Anas et al., 1989
Chaetomium globosum	Hubbard et al., 1982
Chaetomium trilaterale var. *diporum*	Nakashima et al., 1991
Chromobacterium violaceum-C-61	Park et al., 1995
Chrysosporium luteum	Harvey et al., 1995
Cladosporium cladosporioides	Boland and Hunter, 1988

(continued)

Table 19.9.1 (continued)

Antagonist	References
Coniothyrium minitans	Campbell, 1947; Huang et al., 2005a
C. olivaceum	Ivancia, 1992
Cyanobacteria spp.	Kulik, 1995
Dictyosporium elegans	Adams, 1989
Dreschlera sp.	Boland and Inglis, 1989
Enterobacter cloacae	Nelson and Craft, 1991
Epicoccum nigrum	Zhou and Reeleder, 1989
E. purpurescens	Zhou and Reeleder, 1989
Erwinia herbicola	Yuen et al., 1991
Fusarium graminearum	Boland and Inglis, 1989
F. heterosporum	Boland and Inglis, 1989
F. lateritium	Sitepu and Wallace, 1984
F. oxysporum	Ivancia, 1992; Rodriguez et al., 2006
F. solani	Gupta and Agarwal, 1988
Gliocladium virens	Tu, 1997; Das et al., 2002
Gliocladium catenulatum	Huang, 1978; Tu, 1980
G. diliquescens	Huang, 1978; Tu, 1980
G. roseum	Huang, 1978; Tu, 1980; Zhang-Yong Hua et al., 2004
G. virens	Tu, 1980
Glomus etunicatum	Gotoechan, 1999
G. interaradices	Gotoechan, 1999
Hormodendrum spp.	Makkonen and Pohjakallio, 1960
Laterispora brevirama	Ayers and Adams, 1985
Microsphaeropsis carbonacea	EL-Tarabily et al., 2000
Microsphaeropsis centaureae	Watson et al., 1974
Microsphaerpsis ochracea	Carisse, 2001
Monacrosporium janus sp. nov.	Li-Shi Dong et al., 2003
Mucor spp.	Makkonen and Pohjakallio, 1960
Myrothecium verrucaria	Boland and Inglis, 1989
Nectria inventa	Boland and Inglis, 1989
Paecilomyces lilacinum	Singh, 1991
Pantoea agglomerans	Fernando et al., 2004
Penicillium spp.	Lee and Wu, 1979
P. citrinum	Akem and Melouk, 1987
P. cyclopium	Singh, 1991b
P. frequentans	Henis and Chet, 1975
P. griseofulvum	Huang et al., 2005a
Penicillum sheari	Singh, 1991b
P. vermiculatum	Wu, 1989
P. nigricans	Ivancia, 1992
Pseudomonas spp.	Pohjakallio and Solomen, 1950
P. cepacia	Upadhyay and Jayaswal, 1992
P. chlororaphis	Fernando et al., 2004
P. fluorescens	Expert and Digat, 1995; Behboudi et al., 2005
P. maltophila	Kohli et al., 2006
P. putida	Expert and Digat, 1995
Pythium oligandrum	Madsen and Neergaard, 1999
Rhizoctonia sp.	Goodman and Burpee, 1991
Rhizopus arrhizus	Gupta and Agarwal, 1988

(continued)

Table 19.9.1 (continued)

Antagonist	References
Saccharomyces cerevisiae	Suzzi et al., 1995
Serratia marcescens	EL-Tarabily et al., 2000
S. polymuthica	Kamensky et al., 2003; Feng and Thaning, 2001
Sepedonium chrysospermum	Zoina et al., 1990
Sporidesmium sclerotivorum	Uecker et al., 1978
Stachybotris spp.	Makkonen and Pohjakallio, 1960
S. lobulata	Ivancia et al., 1998
Staphylococcus sp.	Zhang, 2004
Streptomyces viridodiosticus	EL-Tarabily et al., 2000
Talaromyces flavus	McLaren, 1987
Teratosperma sclerotivorum	Uecker et al., 1980
T. oligocladium	Uecker et al., 1980
Trichoderma spp.	Mercier and Reeleder, 1985
T. hamatum	Henis and Chet, 1975
T. harzianum	Henis and Chet, 1975; Das et al., 2002
T. kaningii	Dos Santos and Dhingra, 1982
T. oligocladium	Uecker et al., 1980
T. polysporum (Tolypocladium niveum)	Luo et al., 1987
T. pseudokoningii	Dos Santos and Dhingra, 1982
T. roseum	Singh, 1991b
T. viride	Jones and Watson, 1969
T. virens	Huang et al., 2005a
Trichothecium roseum	Huang and Kokko, 1993
Urocladium atrum	Li et al., 2003a
Verticillium spp.	Makkonen and Pohjakallio, 1960
V. albo-atrum	Ivancia, 1992
Yeast (Red and white yeasts)	Mercier and Reeleder, 1987
Zygosaccharomyces spp.	Suzzi et al., 1995

Germ tubes of *S. sclerotivorum* penetrate between cells of the rind and cortex of *S. minor* and *S. sclerotiorum* without specialized structures. The hyphae grow in the intercellular spaces in the medullary region of a sclerotium, before growing to the surface of the sclerotium and sporulating, *Sporidesmium sclerotivorum* forms haustoria to obtain nutrients from cortical and medullary cells of the sclerotium, indicating the biotrophic nature of this parasitism (Bullock et al., 1986). Haustoria are metabolically active between 15 and 30 days after inoculation (Bullock et al., 1986) while sporulation occurs 14–35 days after infection of the sclerotium (Uecker et al., 1978). *Sporidesmium sclerotivorum* primarily uses glucose released from sclerotial glucan by host glucanases (Adams and Ayers, 1983). The energy content of mycelium and macroconidia of *S. sclerotiorum* (18,383 and 16,336 J/g, respectively) and sclerotia of *S. minor* (16,487 J/g) are some what lower than reported for other organisms (Adams et al., 1985). Comparison of the economic coefficient for conversion of glucose to mycelium, [(mycelium dry weight per glucose consumed) × 100] indicated that *S. sclerotivorum* is two to nine times more efficient at using glucose in sclerotia than in culture medium (Adams et al., 1985).

After sporulating on the surface of the sclerotium, *S. sclerotivorum* can grow for upto 3 cm through soil to infect a new sclerotium (Adams and Fravel, 1990). The ability of *S. sclerotivorum* to proliferate and grow through soil to infect new sclerotia facilitates the epidemic destruction of sclerotia. In contrast to biocontrol agents used to protect the rhizosphere, destruction of sclerotia by *S. sclerotivorum* occurs regardless of whether the host plant is present or not.

Survival of macroconidia is influenced greatly by soil temperature (Adams, 1987b). Fifty per cent of macroconidia of *S. sclerotivorum* on filter paper buried in soil are killed in less than 38 h at 40°C, in 6 h at 45°C and in 1 h at 50°C, while more than half of those kept at 35°C are viable after 11 days. Macroconidia survives well at temperatures below 35°C. Thus, high soil temperatures may reduce populations of *S. sclerotivorum* in field soils.

Similarly, survival of macroconidia is also influenced by soil matric potential (Adams, 1987b). All macroconidia stored in soil at −0.02 Mpa are viable after six weeks, while only 73 per cent of those at −116 Mpa and 24 per cent of those at −366 Mpa remain viable. *Sporidesmium sclerotivorum* survives in moist and air dried soils at room temperature for 15 months (Ayers and Adams, 1979b). Sclerotia of *S. minor* are not infected by *S. sclerotivorum* at −3,000 Kpa while percent infection increases between −800 and −300 Kpa (Adams and Ayers, 1980).

Isolates of *S. sclerotivorum* differ greatly in growth habits, amount of growth, number of macroconidia produced and ability to parasitize sclerotia of *S. minor* (Adams, 1987a). Growth and sporulation are unrelated to ability to attach sclerotia (Adams, 1987a).

Three factors viz., presence/absence of ascospores, number and distribution of sclerotia and dispersal of sclerotia through harvesting procedures may make it more difficult to use *S. sclerotivorum* successful against *Sclerotinia* spp. that produce large sclerotia since *S. sclerotivorum* will not be able to grow from one sclerotium to another to cause new sclerotial infections. Because of their initial association with the plant, sclerotia are in an aggregated distribution when crop debris is incorporated into the soil (Adams, 1986; Dillard and Grogan, 1985). For the most part, sclerotia maintain this aggregated distribution even with subsequent disking of the field (Adams, 1986). This aggregate distribution is critical to the success of biocontrol. Since *S. sclerotivorum* grows through soil to infect new sclerotia, the aggregated distribution of sclerotia facilitates spread of the biocontrol agent.

19.10.1.1 Field Application of *Sporidesmium*

Soil borne pathogens generally are not uniformly distributed in soil. These occur in aggregates associated with crop debris. The use of *S. sclerotivorum* to control *S. minor* takes advantage of this aggregated distribution. Because *S. sclerotivorum* can grow for up to 3 cm in soil from one sclerotium to another, if one sclerotium in an aggregate becomes infected. All sclerotia in the aggregate will eventually be destroyed has been reported by Adams and Fravel (1990) and Fravel et al. (1992). The relationship between *S. sclerotivorum* and *S. minor* is a predator-prey

relationship, hence the more pathogen sclerotia that are present, the faster the epidemic destruction will occur (Adams et al., 1984). It has even been suggested that one way to control *S. minor* is to add more pathogen sclerotia to the field (Adams and Fravel, 1993).

Although *S. sclerotivorum* parasitizes sclerotia of fungi other than *S. minor*, it may not be useful for control of these pathogens, One key factor may be the distribution of sclerotia. The inoculum density of sclerotia of *S. minor* is 10–100 times greater than that of *S. sclerotiorum* (Adams and Ayers, 1979). Another factor is the relative unimportance of ascospores in the dissemination of *S. minor* compared to the importance of ascospores in dispersal of *S. sclerotiorum*, hence with *S. minor* inoculum is not blown in from untreated areas. Although *S. sclerotivorum* attacks sclerotia of pathogens such as *Sclerotinia fructicola* and *B. cinerea. S. sclerotivorum* may not be a good choice for control of these pathogens, since production of secondary inoculum impacts greatly on the spread of these pathogens.

19.10.1.2 Inoculum Production

The fact that *S. sclerotivorum* behaves as an obligate parasite is both an advantage and a disadvantage. It is advantageous since its host specificity greatly reduces the possibility of undesirable, non-target effects. *Sporidesmium sclerotivorum* is also reproductively dependent on the pathogen, *S. sclerotivorum* can not proliferate in soil unless it parasitizes sclerotia to obtain energy for reproduction. Thus, *S. sclerotivorum* efficiently colonizes and destroys near by sclerotia.

The biotrophic nature of *S. sclerotivorum* becomes a disadvantage when production of inoculum of *S. sclerotivorum* is done on a commercial scale. *Sporidesmium sclerotivorum* grows very slowly on a glucose-casamino acid medium adjusted to pH 5.5 (Ayers and Adams, 1983; Barnett and Ayers, 1981). The medium also contains KH_2PO_4, $CaCl_2$, ferric-potassium salt of EDTA (FeEDTA), vitamins (biotin and thiamine) and minor elements (B, Mn, Zn, Cu, and Mo) which are needed for a reasonable level of growth. Succinic acid (0.2 per cent) buffers the medium from a rapid drop in pH. Growth rate of the fungus is not affected by the concentration glucose supplied (0.5–2 per cent), although total mycelial yield is dependent on the amount of glucose present (Ayers and Adams, 1983). To provide the surface area needed for sporulation, *S. sclerotivorum* can be cultivated on vermiculite soaked in the liquid medium (Ayers and Adams, 1983). A study of the energy efficiently of *S. sclerotivorum* released the most efficient conversion of energy from the sclerotium to macroconidia of *S. sclerotivorum* occurred with the greatest surface area to substrate ratio (Adams et al., 1985).

19.10.1.3 Compatibility with Fungicides

In order for *S. sclerotivorum* to be useful under commercial production conditions, it must be compatible with commonly used pesticides. *In vitro*, five fungicides

(benomyl, captafol, chlorothalonil, thiabendazole and thiophanate methyl) and one herbicide (naptalam + dinoseb) are toxic at 1 μg/ml to *S. sclerotivorum* (Adams and Wong, 1991). Three additional fungicides (anilazine, pentachloronitrobenzene and thiram) are toxic at 10 μg/ml. Thirty seven additional pesticides are only slightly toxic (100 μg/ml). In soil, chlorothalonil at 10 μg/g soil prevents infection of sclerotia of *S. minor* by *S. sclerotivorum*. In a soil column experiment designed to simulate field conditions including irrigation, benomyl, chlorothalonil, Iprodione, procymidone and Vinclozolin does not affect *S. sclerotivorum* in concentrations likely to be encountered in the field.

The impact of possible parasites to *S. sclerotivorum* on its ability to reduce populations of sclerotia must also be elucidated before *S. sclerotivorum* is widely used for disease control. *Laterispora brevirama* is morphologically similar to *S. sclerotivorum* and *T. oligocladium* but *L. brevirama* does not attack sclerotia as do *S. sclerotivorum* and *T. oligocladium. Laterispora brevirama* colonizes and proliferates on sclerotia that have been previously attacked by *S. sclerotivorum* or *T. oligocladium* (Ayers and Adams, 1985).

Laterispora brevirama forms specialized contact cells on hyphae of *S. sclerotivorum* and *T. oligocladium*, but does not invade these fungi. *Laterispora brevirama* added to soil at the same concentration as *S. sclerotivorum* does not affect the rate of destruction of sclerotia of *S. minor* by *S. sclerotivorum*, however, the number of new macroconidia produced by *S. sclerotivorum* is reduced (Ayers and Adams, 1985). The exact role of *L. brevirama* needs to be clarified to determine if *L. brevirama* is a direct parasite of *S. sclerotivorum* and *T. oligocladium* or if it is a secondary parasite of sclerotia. The long term effects of reduced sporulation of *S. sclerotivorum* due to *L. brevirama* need to be determined.

19.10.2 Biological Control Strategies for Sclerotinia *Diseases*

Biological control strategies for any disease are basically based to target different stages in the disease cycle as follows.

19.10.2.1 Reduction of Initial Inoculum

The most important strategy for biological control of diseases caused by *Sclerotinia* spp. is to reduce the concentration of initial inoculum by killing sclerotia or inhibiting their germination. Numerous authors have screened microorganisms recovered from soil, sclerotia, or other habitats for antagonism as well as for parasitism of sclerotia to identify promising biological control agents (Table 19.9.1).

Numerous methods have been used to isolate mycoparasites or antagonists of *Sclerotinia* spp., including baiting with mycelium, baiting with sclerotia and direct isolation from field collected sclerotia (Sandys-Winsch et al., 1994). In general,

field assays often are too impractical or expensive for screening large numbers of isolates (Andrews, 1992; Sandys-Winsch et al., 1994). Screening of potential BCAs on living plant tissues or in soil, instead of on nutrient-rich media, often provides a more accurate prediction of performance in field environments (Whipps, 1987).

Considerable Attention has been placed on the use of parasitic fungi, or mycoparasites to reduce the number of sclerotia in infested soils. These mycoparasites can weaken or kill sclerotia and thereby reduce the amount of initial inoculum available for an epidemic. One of the most effective of these mycoparasites is *Sporidesmium sclerotivorum* (Adams and Fravel, 1993; Ayers and Adams, 1981a). This obligate mycoparasite has numerous properties that contribute its success as a BCA, perhaps one of the most important being its ability to grow through soil and parasitize sclerotia e.g., *S. sclerotivorum* parasitized sclerotia of *S. minor* in natural soil and destroyed more than 95 per cent of sclerotia within ten weeks (Ayers and Adams, 1979a, b). Similarly, lettuce drop, caused by *S. minor* is suppressed by up to 83 per cent when 1×10^3 conidia of *S. sclerotivorum* per gram of soil are applied (Adams and Ayers, 1981).

Another promising mycoparasite of *S. sclerotiorum* is *Coniothyrium minitans* (Ghaffar, 1972; Huang and Kokko, 1987; Phillips and Price, 1983; Tu, 1984). *Coniothyrium minitans* is an endemic and soil borne fungus in many regions of the world that can penetrate the cell walls of both hyphal and sclerotial cells by physical pressure, or by a combination of physical and enzymatic action (Huang and Kokko, 1987; Phillips and Price, 1983; Tu, 1984). Hyphae of *C. minitans* can grow inter and intracellularly within sclerotia and eventually cause destruction and disintegration of sclerotial tissues. Pycnidia develop both on and inside these infected tissues. This mycoparasite has been examined in several strategies of biological control of *S. sclerotiorum*, including reduction of initial inoculum in the soil and reduction of secondary inoculum in the phyllosphere. In greenhouse trials, preplant soil-incorporations of different solid substrate inocula of *C. minitans* reduce sclerotial populations and controls lettuce drop (*S. sclerotivorum*) is a sequence of crops. More than 74 per cent of the recovered sclerotia are parasitized by the mycoparasite (McQuilken and Whipps, 1995). Sclerotia of *S. sclerotiorum* infects by *C. minitans* forms stipes and apothecia less frequently than non-infected sclerotia (Trutmann et al., 1982).*C. minitans* penetrates into mycelium of *S. sclerotiorum* that subsequently collapses and becomes necrotic (Whipps and Gerlagh, 1992). The production of β-1, 3 glucanases and chitinases enable the mycoparasite to utilize the host cells and mycelium of the mycoparasite proliferates around dead hyphae of the host fungus. *C. minitans* is a very potential parasite of sclerotia of *S. sclerotiorum*, reducing the survival of sclerotia in field trials by about 90 per cent (Gerlagh et al., 1995b).

Trichoderma spp., such as *Trichoderma koningii*, *Trichoderma harzianum* and *Trichoderma viride*, also has been used to reduce the number of viable sclerotia in soil. These are an important group of fungi with biological control potential and are being examined in numerous patho-systems for efficacy. In soil infested with one isolate of *T. koningii* at 10^8 conidia/g, 100 per cent of the sclerotia of *S. sclerotiorum* are parasitized and killed within 60 days under field conditions (Dos Santos and

Dhingra, 1982), although selected isolates of the same species varies in their efficacy. *Trichoderma harzianum* parasitizes mycelium and sclerotia of *S. sclerotiorum* and destroys sclerotia within 15 days (Singh, 1991b). *Trichoderma roseum* reduces the viability of sclerotia by upto 54 per cent after 15 days of incubation in soil. None of the sclerotia can germinate after 30 days of incubation, even though this fungus does not appear to parasitize *S. sclerotiorum*. Other fungi such as *Penicillium* spp. and *T. harzianum* are both antagonistic and parasitic to *Sclerotinia* spp. (Singh, 1991a).

The timing of application of BCAs is an important factor in determining their efficacy for reducing the initial inoculum of *Sclerotinia* species. Application of *T. koningii* as a pre-sowing treatment for *S. sclerotiorum* in spring and summer results in significant reductions in viability of sclerotia while its application as a post-harvest soil treatment in winter is not effective (Trutmann and Keane, 1990). Similar results are obtained when *C. minitans* is used (Trutmann et al., 1980). Applications of *C. minitans* in the fall in field conditions result in negligible numbers of viable sclerotia after one month, however, a winter application of *C. minitans* does not result in significant infection of sclerotia or in a reduction of sclerotial viability.

Some soil animals (e.g., Collembola, fungus gnats) can weaken and consume sclerotia in soil or act as vectors carrying mycoparasites from one sclerotium to another, or both. Some of these animals play an important role in reducing the number of sclerotia in soil (Anas and Reeleder, 1988a; Anas et al., 1989; Whipps and Budge, 1993). Larvae of fungus gnats (Diptera: Sciaridae) consume sclerotia of *S. sclerotiorum* infected with *C. minitans* (Turner and Tribe, 1976). They also graze uninfected sclerotia, removing the protective rind and predisposing them to infection by *Trichoderma* spp. (Anas and Reeleder, 1988a). Transmission of mycoparasites by collembola also has been observed (Whipps and Budge, 1993). Combinations of animals and mycoparasites may have synergistic effects that can be used to improve the control of these diseases. However, additional studies, especially field studies are needed to clarify the role of these animals in the survival of sclerotia.

Antagonistic bacteria also have been studied as potential BCAs of sclerotia in soil. Application of *Bacillus* strains in soil reduces apothecia formation by *S. sclerotiorum* to 36 per cent as compared to the number of apothecia formed in the untreated control. Similarly, oilseed rape yield losses are reduced by application of a bacterial strain (Luth et al., 1993). Bacteria may be more effective at protecting the root or crown of susceptible plants from infection by myceliogenically germinated sclerotia than reducing the production of ascospores by the pathogen.

Many mycoparasites produce a significant reduction in the number of viable sclerotia in soil, but rarely have these results been correlated with effective disease control or if effective the level of disease control is low. Furthermore, quantitative relationships between the prevalence of inoculum and resulting disease are poorly understood and appear to vary considerably among locations and years (Boland and Hall, 1988a, b). Selected biological control treatments may be effective at reducing

the number of sclerotia or apothecia in infested soils, but reductions in disease are often not observed because there is still sufficient inoculum in infested areas to initiate epidemics.

19.10.2.2 Reduction of Secondary Spread of Inoculum

Sclerotia produced in one growing season become an additional source of inoculum for the following season or crop and thereby increase the severity of disease. Several investigations have examined the potential for controlling disease through applications of mycoparasites to mature plants to reduce the number of viable sclerotia formed on and in diseased tissues and debris. *Coniothyrium minitans* parasitizes and kills sclerotia of *S. sclerotiorum* produced on the inside and outside of sunflower roots and the inside of stems (Huang and Hoes, 1976). Foliar applications of *C. minitans* within the growing season reduces the number of sclerotia produced on diseased plants and results in low carpogenic viability of those sclerotia that are produced but fails to suppress white mold of bean (Trutmann et al., 1982).

Applications of spore suspensions of BCAs to the phyllosphere, to reduce the number of viable sclerotia in subsequent crops may be a more suitable strategy for *Sclerotinia* diseases that do not involve ascospores in the disease cycle. This approach has been used successfully to manage lettuce drop incited by *S. minor* (Adams and Fravel, 1990). *Sporidesmium sclerotivorum* is applied to diseased lettuce plants at the rate of 0.2, 2 and 20 kg/ha at harvest so that the mycoparasite may be in contact with sclerotia of the pathogen. Plots with *S. sclerotivorum* shows significantly lower disease incidence than the non-treated plot in the first, second and third crops. The highest lettuce drop reduction was observed upto 72 per cent (Adams and Fravel, 1990).

19.10.2.3 Prevention of Infection in the Rhizosphere

In some *Sclerotinia* diseases, the pathogen survives in the soil and attacks the plant roots or crown, this results in a root rot or plant wilt, or both. Protection of these plant tissues from infection by the pathogen has been used to control these diseases. One such approach is seed bacterization. Seed treatment with *Pseudomonas fluorescens* and *Pseudomonas putida* (1×10^6 bacteria/seed) significantly protects sunflower from early injury to the root and collar (Expert and Digat, 1995). *Pseudomonas cepacia*, strains J82 rif and J5 rif are applied as seed treatments at 2×10^8 cells/seed which results in increased sunflower emergence in the field in the presence of *S. sclerotiorum*. Although three antifungal compounds have been identified from *P. cepacia*, strain J82rif, two antibiosis negative mutants are similar to their parent strains in increasing sunflower emergence in a growth chamber experiment (Mc Loughiin et al., 1992). This strategy also may be used to control lettuce drop caused by *S. minor*. Treatment of lettuce seedlings by dipping in a solution of

antagonistic bacteria before transplanting may protect the crops from infection by the pathogen in the field.

19.10.2.4 Prevention of Infection in the Phyllosphere

Colonization of senescent leaves and flowers is an important preliminary step for infection by *S. sclerotiorum* in many susceptible hosts. Therefore, prevention of this colonization stage may protect plants from disease.

Numerous microorganisms have been evaluated for the potential to suppress *S. sclerotiorum* in the phyllosphere by interfering with the colonization of infection sites. *Epicoccum nigrum* (syn. *Epicoccum purpurascens* Schol-Schwarz, Cannon, 1986) has shown particularly promising results. In greenhouse conditions, an application of conidial suspensions (1×10^6 conidia/ml) of *E. nigrum* suppresses the incidence of lettuce drop by 46.7 per cent (Mercier and Reeleder, 1987). With multiple applications (two to four times) of conidial suspensions during crop flowering, the severity of white mold of bean is suppressed by upto 55.2 per cent (Zhou and Reeleder, 1989). Disease control with *E. nigrum* is similar to application of the fungicide, iprodione.

Fungi such as *E. nigrum* are considered to be primary saprotrophic colonizers of phyllosphere and senescing tissues (Hudson, 1971). The mechanism (s) of action of *E. nigrum* in suppressing disease caused by *S. sclerotiorum* has not been elucidated, but several factors seem to be responsible. *Epicoccum nigrum* has pigmented multicellular conidia and these features are considered as protective mechanisms against desiccation and strong sunlight (Nicot, 1960). Conidia germinates at relative humidity as low as 92 per cent and under more favorable conditions such as 100 per cent relative humidity, grow faster than other fungal colonizers of plant tissues and has a shorter latent period before germination than competitors (Hudson, 1971). *Epicoccum nigrum* is one of several fungi with high cellulolytic activity (Siu, 1951). Furthermore, conidia of *E. nigrum* does not require exogenous nutrients for germination (Hudson, 1971), a beneficial attribute for microorganisms colonizing the phyllosphere.

Rapid colonization of bean flowers by *E. nigrum* is an important component of suppression of white mold (Zhou and Reeleder, 1991; Zhou et al., 1991). Colonized, *E. nigrum* protects bean flowers from infection or colonization by the pathogen and the production of antifungal compounds also in part by competition for nutrients.

Alternaria alternata and *Cladosporium cladosporiodes* are the most prevalent filamentous fungi recovered from rapeseed and bean flowers in all stages of flower development. These are associated with significant disease suppression in the early stages of petal development and senescence in bean (Boland and Inglis, 1989; Inglis and Boland, 1990). Competition for nutrients in the infection court of senescing flowers and antibiosis both appears to be responsible for suppression of disease (Boland and Hunter, 1988; Inglis and Boland, 1992).

Bacteria also have been evaluated for the suppression of disease in the phyllosphere. Pretreatment of bean flowers with selected strains of *Erwinia herbicola* and

Bacillus polymyxa prevents the colonization of flowers by *S. sclerotiorum* and the subsequent development of white mold (Yuen et al., 1991). Insufficient epiphytic survival and colonization are a common limitation of bacterial antagonists, particularly in field conditions when disease pressure is moderate to high (Yuen et al., 1991). Bacteria are considered to be early success ional colonizers of the phyllosphere (Andrews, 1992; Blakeman, 1985) and their efficacy as BCAs is strongly influenced by factors such as environmental conditions, the transience of petals, cropping practices and so on. Furthermore, bacteria primarily colonize the surface of petals in comparison to many filamentous fungi that colonize the entire petal tissues, essentially preventing them from acting as infection sites for germinating ascospores of *S. sclerotiorum* (Inglis and Boland, 1990).

Integration of chemical and biological controls may provide a more consistent suppression of disease by moderating populations of the pathogen and other factors to provide conditions more favourable for efficacy of the biological control agent.

Suppression of disease by many of the agents is most effective under environmental conditions that are least conducive to disease, however, the efficacy of one agent, *E. nigrum* is comparatively independent of environment and suppresses disease by 100 per cent in all environments. Antibiosis may be involved as a mechanism of action for this BCA.

Strain improvement can be achieved through selection and genetic manipulation of selected BCAs for improved colonization of the phyllosphere, or other characters important to biological control. After exposure to UV radiation, selected strains of *E. nigrum* display improved sporulation, resistance to a fungicide and improved efficacy in suppression of white mold (Zhou and Reeleder, 1990).

The integrated control of diseases caused by *Sclerotinia* spp using BCAs in combination with fungicides has been used as another strategy to enhance disease control. The combined treatment of *A. alternata* and benomyl suppresses the incidence of white mold more than benomyl alone (Inglis and Boland, 1992).

The efficacy of BCAs also can be increased with improved formulations. The addition of malt extract in spore suspensions applied to bean plants improves conidial germination of *E. nigrum* on flowers and increases mycelial coverage on emerging flowers. Also, the type and ratio of nutrients provided to *E. nigrum* influences the inhibition of *S. sclerotiorum* and the production of antifungal compounds (Zhou et al., 1996).

The use of bees for vectoring inoculum of BCAs (Sutton and Peng, 1993) to flowering plants may be a feasible approach for management of diseases caused by *S. sclerotiorum* in selected crops (Israel and Boland, 1992). Formulations are an important factor affecting bee behaviour, efficacy and storage of the BCAs, but selected formulations are deposited at sufficient concentrations on flowering canola plants to suppress disease. This approach has considerable potential as a method of vectoring BCAs onto flowering crops that are naturally attractive to foraging bees (e.g., canola) but has limited potential for other crops that are not as attractive to bees (e.g. bean, soybean).

19.10.2.5 Reduction of Virulence

Hypovirulence refers to the reduced virulence of selected isolates within a population of a plant pathogen. In many cases, hypovirulence has been associated with the presence of double-stranded ribonucleic acids (dsRNA) characteristics of fungal viruses (Nuss and Koltin, 1990) and at least three of these agents from *Cryphonectria parasitica*, the causal agent of chestnut blight, have been classified within the new virus family, Hypoviridae (Hillman et al., 1995), however, other factors such as mitochondrial mutations (Mahanti et al., 1993), nuclear mutations and encapsidated fungal viruses have been associated with hypovirulent isolates. Therefore, hypovirulence is best considered as a phenotypic response characterized by reduced virulence but also may be associated with other characters such as reduced growth rate, sporulation, survival and so on. The potential in utilizing hypovirulent isolates of fungal pathogens in a biological control strategy resides in the ability to transfer hypovirulence from hypovirulent isolates to virulent isolates and thereby, reduce the mean disease severity of the population through overall reductions in virulence, growth, sporulation and/or survival.

Hypovirulent isolates of *S. sclerotiorum* and *S. minor* with their role in reducing virulence in populations of these pathogens and their potential application in disease management have been identified. One isolate of *S. sclerotiorum* has been reported that is hypovirulent and contains dsRNA (Boland, 1992). This isolate grow slowly in culture, develops an a typical colony morphology, produces significantly smaller lesions on celery than virulent isolates and contains dsRNA. The hypovirulent phenotype and dsRNA are transferred to vegetatively compatible recipient isolates through hyphal anastomosis and recipient isolates develop the hypovirulent phenotype and become hypovirulent.

Ultra structural examination of hyphal and sclerotial cells from the virulent isolate of *S. sclerotiorum* reveal pleomorphic vesicles surrounded by a double-membrane associates with the nuclear envelope (Boland et al., 1993). No such vesicles are found in a near-isogenic, virulent, dsRNA free isolate. The evidence indicates that dsRNA in this hypovirulent isolate is not associated with virus particles, but instead is surrounded by a lipid membrane. This suggests that this agent may be a member of the newly established family of unencapsidated dsRNA viruses termed Hypoviridae (Hillman et al., 1995).

Despite the discovery of a hypovirulent isolate of *S. sclerotiorum* that can transmit hypovirulence to selected, virulent isolates, further attempts to utilize hypovirulent isolates of this pathogen for biological control have not been actively pursued because of the known diversity of vegetative (Ford et al., 1995) and mycelial compatibility groups (Kohli et al., 1992; Kohn et al., 1991) within this pathogen, however, the number of MCGs is *S. minor* is relatively small (Patterson, 1986) and therefore, the use of transmissible hypovirulence in this pathogen represents a more promising approach as a management strategy for disease in controlled environments. Mycelial suspensions of a hypovirulent isolate of *S. minor* applied to leaf lesions initiate by compatible isolates on lettuce suppresses lesion expansion upto 100 per cent and reduces the development of sclerotia on diseased tissues upto 100

per cent (Melzer and Boland, 1996). The earlier in lesion development the hypovirulent isolate is applied, the more it arrests the growth of the lesion of the virulent isolate and reduces the numbers of sclerotia produced. Sclerotia that are produced on leaves treated with the hypovirulent isolate tends to be nonviable, or to grow a typically. Application of the hypovirulent isolates to lesions initiated by incompatible, virulent isolates does not suppress disease or reduce the development of survival structures. The results suggest that hypovirulent isolates of *S. minor* applied to actively growing lesions on lettuce plants in the field may reduce inoculum production and increase the level of hypovirulence in the population.

19.11 Integrated Disease Management

It is now very evident that several strategies must be combined into a single management programme in order to achieve maximum disease control. Crop rotation and weed control in non-host crops are essential for preventing the soil population of sclerotia from increasing and reducing the crop density and planting crops with open canopy structures are effective ways to minimize disease development (Blad et al., 1978; Coyne et al., 1974; Kruger, 1980; Morrall and Dueck, 1982; Steadman, 1979; Williams and Stelfox, 1980b). Foliar sprays such as Benomyl and soil fungicides such as calcium cyanamide are giving good results in the field on several crops.

Sclerotinia rot of sunflower can be controlled with integrated approach (Table 19.11.1) of pre-sowing flooding for 30 days, seed treatment and spray with carbendazim (0.2 per cent) and *T. harzianum*, soil amendment (Singh and Tripathi, 1997).

An integrated control measure for *Sclerotinia* of sunflower include a crop rotation of more than two years, delayed sowing (19–25 May), increased application of K and sprays of 50 per cent Sumitex (procymidone) under China conditions (Hua et al., 1994). The integrated effect of vermicompost, soil solarization, herbicide (EPTC), fungicide (procymidone), *Trichoderma harzianum* and *Bacillus subtilis* have been evaluated for the control of *S. sclerotiorum* by Pereira et al. (1996). Soil solarization through coverage of transparent polythene (0.1 mm) for 45 days is a good control strategy. EPTC treatment significantly increases the degree of control irrespective of the depth of the sclerotia in the soil. Similarly, *T. harzianum* in the presence of vermicompost combined with EPTC treatment is a most promising control strategy for *S. sclerotiorum*. Soil solarization combined with procymidone is the best for the control of *S. minor* drop in lettuce (Sinigaglia et al., 2001).

Seed treatment, rotation between vegetable and cereal crops, fertilizers rational, close planting, pruning of old and infected leaves and 50 per cent carbendazim are used as integrated disease control measures for the control of rape *Sclerotinia* rot in Shanxi, China (Yu et al., 1995). Seed treatment and foliar spray of *Allium sativum* extract provides good control (70.2 per cent) of

Table 19.11.1 Integrated management of *Sclerotinia* rot of sunflower under screen house and field conditions (Adapted from the publication of Singh and Tripathi, 1997. With permission)

	Disease incidence (%)			
Treatment	Screen house		Field	
Seed treatment (ST)	–		36.3[a]	(30.9)[b]
Spray (SP)	–		31.6	(34.2)
Antagonists (AT)	–		30.1	(33.3)
ST + SP	9.2	(17.7)	15.0	(22.8)
ST + AT	6.7	(15.0)	13.3	(21.4)
SP + AT	12.0	(18.4)	25.4	(30.3)
ST + SP + AT	3.1	(10.0)	10.0	(18.4)
Flooding (FL)[c]	7.3	(15.7)	10.7	(19.0)
FL + ST	3.7	(11.0)	5.3	(13.3)
FL + SP	4.6	(12.4)	6.7	(14.9)
FL + AT	3.8	(11.3)	5.7	(13.8)
FL + ST + SP	0.0		4.3	(12.0)
FL + ST + AT	0.0		3.0	(10.0)
FL + SP + AT	0.0		5.3	(13.3)
FL + ST + SP + AT	0.0		0.0	
Control	39.0		40.0	(38.6)
CD at 5%	3.72		1.77	

[a]Average of three replications
[b]Figures in parentheses are angular transformed value
[c]Flooding was done for 30 and 15 days continuously in screen and field, respectively

Sclerotinia rot of Indian mustard with higher yield (34.3 per cent) (Chattopadhyay et al., 2004).

For management of white mould of beans in Ontario, Canada, Tu (1989c) described an effective integrated control programme. It involves seed treatment with DCT (diaznon 6 per cent, Captan 18 per cent, thiophanate-methyl 14 per cent) to prevent the introduction of seed borne *S. sclerotiorum* to disease free fields and the use of resistant cvs Ex Rico 23, Crestwood and Centralia. However, a well executed integrated control can reduce the disease significantly and keep the yield loss to a minimum. Integration of pre-sowing application of carbendazim granules @ 10 kg/ha^{-1}, seed treatment with 1:1 combination of carbendazim + Thiram @ 2.5 g/kg^{-1} seed and three fortnight sprays starting with the initiation of flowering of carbendazim 50 WP @ 0.1 per cent gives excellent (97.7 per cent) control of white rot of pea (Table 19.11.2) with five fold increase in yield (Sugha, 2001). GR isolate of *T. viride*, bulb extract of *A. sativum* and soil application of K results in significantly higher test weight and oil content of mustard seeds (Chattopadhyay et al., 2001). An IDM module (Table 19.11.3) for management of *Sclerotinia* rot of mustard (Singh, 2001) and carrots (Fig. 19.11.1) has been suggested (Kora et al., 2003). The integrated control measure includes the following items (Tu, 1997).

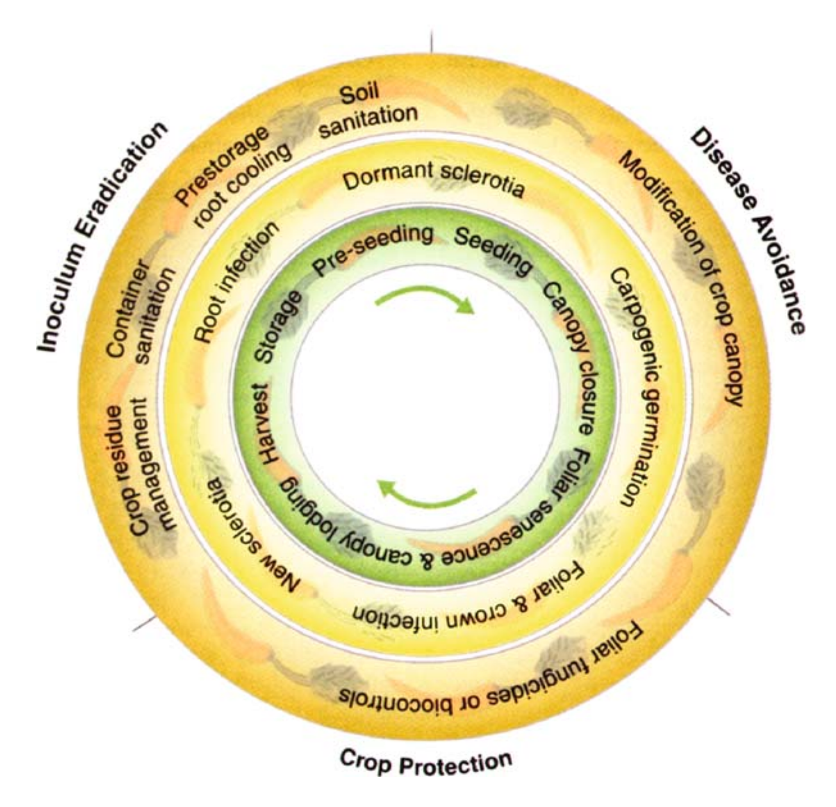


Fig. 19.11.1 An integrated model for managing *Sclerotinia* rot of carrot that incorporates three disease management principles and selected disease control strategies (outer circle), that target particular stages in the life cycle of *Sclerotinia sclerotiorum* (middle circle) or development of carrot crop (inner circle). Control strategies corresponding to respective stages are indicated by positional overlap (Adapted from the publication of Kora et al., 2003. With permission) (*See Color Plates*)

19.11.1 Site Selection

The degree of field infestation by *S. sclerotiorum* varies greatly. Fields with a previous history of severe white mould should be planted with resistant crops. However, mono cropping in sunflower declines wilt (Huang and Kozub, 1991b).

19.11.2 Crop Rotation and Zero Tillage

A combination of proper crop rotation with zero tillage in the fields with a history of white mould reduces risk.

Table 19.11.2 Effect of integration of soil application of carbendazim granules, seed treatment with Bavistin + Thiram and foliar sprays of Bavistin on the incidence of white rot of pea (Adapted from the publication of Sugha, 2001. With permission)

Treatments	Disease incidence (%)	Disease control (%)	Green pods plot^{-1} (kg)
T_1 (Carbendazim granules at sowing in furrows)	18.5 (25.47)	76.5`	5.0
T_2 (T1 + seed treatment with Bavistin + Thiram (1:1))	12.3 (20.53)	84.4	5.8
T_3 (Seed treatment with Bavistin + Thiram (1:1))	15.1 (22.87)	80.9	5.5
T_4 (Foliar sprays of Bavistin – 0.1%)	8.7 (17.15)	89.0	6.1
T_5 (T1 + T4)	6.1 (14.30)	92.3	6.5
T_6 (T2 + T4)	1.8 (7.71)	97.7	7.5
T_7 (T3 + T4)	5.5 (13.56)	93.0	6.9
T_8 (Control)	78.9 (62.65)	–	1.3
CD (P = 0.05%)	(7.91)		1.38

Angular transformed values in parentheses

Table 19.11.3 An IDM module for the management of *Sclerotinia* rot of mustard (Adapted from the publication of Singh, 2001. With permission)

Crop stage	Management practices
Pre-sowing	Proper field sanitation, removal of debris from previous crop, summer ploughing of the fields to kill the spores per residual population of the insect pests should be done.
Sowing	Use of disease tolerant varieties, selection of sclerotia free seeds. Planting between 15–25 Oct. optimum, early sowing should be avoided. Seed treatment with Benomyl @ 0.1%.
	Planting on raised beds recommended, avoidance of narrow spacing, avoidance of heavy seed rate.
Seedling and vegetative stage	Practices for reducing moisture retention in the canopy and promoting aeration to be adopted.
	Irrigation timing also play an important role hence minimum timely irrigation to be given.
Flowering stage	Spraying the crop with fungicides such as benomyl or thiophanate methyl during flowering. Protection is necessary because of the petals play a critical role in infection, fungicide application to be done when most of the plants have reached 20–30% bloom.

19.11.3 Seed Treatment

In the infected bean seeds the pathogen is able to survive as a dormant mycelium in testae and cotyledons for three years or longer (Tu, 1988). Captan and thiophanate-methyl used in seed treatment are 100 per cent effective in eradicating the fungus from the infected seeds (Tu, 1989c). In sunflower, seed treatment with fungicides (Table 19.11.3.1) significantly reduces the incidence of early root and basal stem infections (Rashid and Swanson, 2001).

19.11.4 Resistant Cultivars

Cv. Ex Rico-23 has gained world-wide acceptance as a main source for genetically resistance against white mould disease. The list of resistant varieties have been discussed in Chapter 18.

Table 19.11.3.1 Effects of seed treatment in sunflower on early infections by *Sclerotinia sclerotiorum* and on yield (Adapted from the publication of Rashid and Swanson, 2001. With permission)

	1998		1999		2000	
	Control (%)		Control (%)		Control (%)	
Treatment	Stand	Yield	Stand	Yield	Stand	Yield
Control	100	100	100	100	100	100
Control + *Sclerotinia*	36[a]	40[a]	31[a]	22[a]	16[a]	20[a]
Topsin	122[b]	180[b]	109	39[a]	140[b]	165[b]
Topsin + *Sclerotinia*	66[b]	78	46[b]	32	38[b]	63[b]
Ronilan	NT	NT	135[b]	176[b]	156[b]	–
Ronilan + *Sclerotinia*	NT	NT	85[b]	110[b]	75[b]	115[b]
Quadris	NT	NT	136[b]	123	147[b]	143[b]
Quadris + *Sclerotinia*	NT	NT	41	44	18	37
Maxim	NT	NT	NT	NT	159[b]	161[b]
Maxim + *Sclerotinia*	NT	NT	NT	NT	83[b]	91[b]
LSD (P = 0.05%)	20	57	13	57	19	35

[a]Significantly more diseased than the checks

[b]Significantly better than the checks

Compare the fungicide seed treatments to the control; and the fungicide seed treatments + *Sclerotinia* to the control + *Sclerotinia* treatment

19.11.5 Plant Type

In general, upright types of plants have less severe white mould than bush or viny types (Saindon and Huang, 1992). Compact bush types have more mould than open bush types. Bean plants with indeterminate growth habits often have more severe white mould disease than those with determinate ones (Coyne et al., 1974; Schwartz et al., 1978). Plants that have a large and dense canopy with leaves close to the soil maintain a wet microclimate longer than plants that have a small and thin canopy with upright characteristics. Consequently, the former type of plants has a higher incidence and more severe white mould than the later. To avoid white mould, selection of plant types with upright characteristics and thin canopy that allow adequate penetration of sunlight and under the canopy aeration is important.

19.11.6 Row Width and Plant Density

Narrow row and high plant density reduce air circulation and trap moisture in the canopy. Thus, they contribute to higher incidence and more severe white mould than conventional row width and reduced plant density. Narrow row and high plant density not only increase early senescence but also increase contact of plant parts. Increase in senescence and in contact of plant parts promote infection and spread of white mould, respectively. Air circulation between rows of beans can be improved by planting the rows parallel to the prevailing winds by reducing the seeding rate and by practicing stringent weed control. However, reducing the seeding rate often reduces the bean yield as well. Keeping constant space between rows 0.5 m, reduction of planting densities from 15 plants/m to 5 plants/m decreases white mould incidence and severity and increases yield of dry beans (Vieira et al., 2001).

19.11.7 Chemical Control

Amongst several chemicals tested, fungicides like benomyl, chlorothalonil, thiophanate-methyl, iprodione and dichloran are the most effective. Proper timing of spray and method of application have a great impact on results. Aerial application by aircraft is relatively ineffective as compared with ground application with boom sprayer. The later gives better penetration of spray into the crop canopy. Two application of fluazinam increases dry bean yield by 118 per cent (Vieira et al., 2001).

19.11.8 Biological Control

The time to implement biological control is in the resting (or sclerotium) stage of *S. sclerotiorum*, during which the pathogen has little mobility, or at the germinating stage, during which the pathogen is most vulnerable to attack. *Coniothyrium minitans* and *Gliocladium virens* have shown practical potential for biological control of *S. sclerotiorum* (Budge et al., 1995). *C. minitans* applied to soil as solid-substrate inoculum can infect sclerotia of *S. sclerotiorum* year round and effectively reduce their number and viability in the soil (Budge et al., 1995; Gerlagh et al., 1994; Huang, 1977). Foliar application with spore suspension of *C. minitans* has been shown to reduce disease severity. The applications of spore suspension of *C. minitans* to crop residues occupied by *S. sclerotiorum* may reduce disease carry over and can be used in combination with soil incorporation treatments. Growing the fungus in the plant debris and reintroducing it into the soil can conceivably hasten the destruction of sclerotia in the field (Budge and Whipps, 1991; Trutmann et al., 1982; Whipps and Gerlagh, 1992). The reduction in cases of disease caused by *Sclerotinia* due to the use of Contans WG (*C. minitans*) as soil application in different countries and crops are presented in Table 19.11.8.1 (Luth, 2001a, b).

Contans WG is the preparation formulated as a water dispersible granule containing only glucose as carrier and the purified conidia of *C. minitans*. One gram of product contains 1×10^9 viable conidia (Luth, 2001a). Considering development of resistance in biological agents like *Bacillus subtilis subsp subtilis,* it should form part of integrated disease control (Li and Leifert, 1994). Sodium thioglycolate inhibits the formation of sclerotia in *S. sclerotiorum* but it has no adverse effect on the mycelial growth and pycnidium formation in *C. minitans*. The combined application of both can be used in integrated control of *Sclerotinia* diseases (Dimopoulou et al., 2000). For the control of white rot of pea, sodium alginate pellet formulation (800 No/m^2) followed by soil application of wheat bran based *T. viride* formulation @ 11.2 g/m^2 is

Table 19.11.8.1 Reduction in disease caused by *Sclerotinia sclerotiorum* due to the use of Contans WG (*C. minitans*) in different countries and crops (Adapted from the publication of Luth, 2001a. With permission)

Country	Institution	Crop	Conditions	Rate per ha (kg)	Depth of incorporation (cm)	Percentage of disease reduction (%)
Poland	Research Institute of Vegetable crops in Skierniewice	Lettuce	Glass-house	8	10	79.8
	Institute of Pomology and Floriculture in Skierniewice	Gerbera	Glass-house	4	10	100

(continued)

Table 19.11.8.1 (continued)

Country	Institution	Crop	Conditions	Rate per ha (kg)	Depth of incorporation (cm)	Percentage of disease reduction (%)
		Gerbera	Glass house	4	10	91.6
		Chrysanthemum	Glass-house	4	10	84.2
		Chrysanthemum	Glass-house	4	10	88.6
Spain	Agrichem, S.A.	Iceberg lettuce	Open field	4	20	93.0
			Open field	4	20	89.2
			Open field	6	30	88.6
			Open field	6	30	95.9
Germany	Landespflanzen-schutzamt M-V	Oilseed-rape	Open field	2	5	93.8
	Landesamt f. Ernahrung and Landw, Kiel	Oilseed-rape	Open field	2	5	62.5
Switzerland	OMYA AG	Tobacco	Open field	4	5	93.6

most effective delivery system. *T. viride* population significantly increases (Figs. 19.11.8.1, 19.11.8.2) even after 60 days of application (Kapil and Kapoor, 2005).

19.12 Resistance to Fungicides in *Sclerotinia*

Sclerotinia sclerotiorum wild isolates collected from oilseed rape (*Brassica napus*) plants which had never been treated with carbendazim (MBC) were tested for resistance in laboratory experiments. The minimum inhibitory concentration (MIC) of MBC to these wild isolates is 5 µg/ml, the mean EC50 value (the MBC concentration that reduced mycelial growth by 50 per cent compared with the control) with 95 per cent confidence limits is 0.2198 ± 0.1083 µg/ml. Six MBC-resistant isolates were observed from 86 isolates collected from the oilseed rape breeding experimental field in Zhenjiang Institute of Agricultural Science. The EC50 values of these are all higher than 2,000 µg/ml MBC. It is difficult to measure their MIC value. The resistance to MBC of these isolates is stable through sexual and asexual propagation. Pathogenicity to detached rape leaves is not different between MBC resistant and sensitive isolates of *S. sclerotiorum*. MBC resistant isolates of *S. sclerotiorum* can infect rape leaves soaked in MBC of 1,000 µg/ml concentration, while MBC-

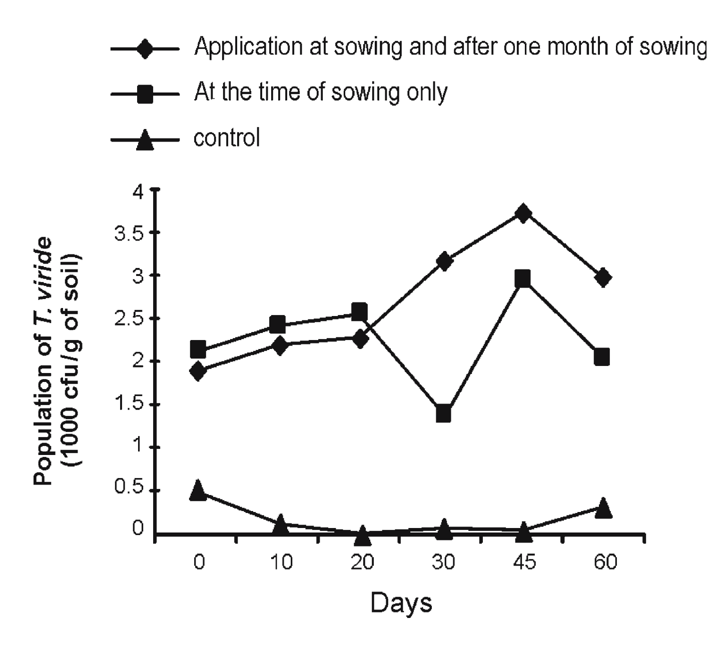


Fig. 19.11.8.1 Population dynamics of *Trichoderma viride* under field conditions (Adapted from the publication of Kapil and Kapoor, 2005. With permission)

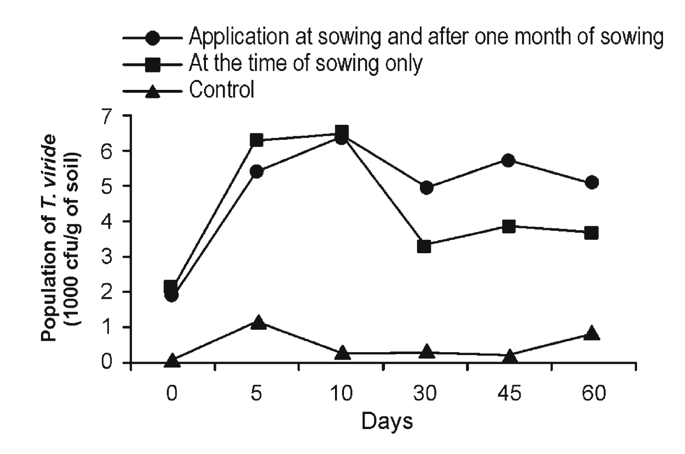


Fig. 19.11.8.2 Population dynamics of *Trichoderma viride* under field conditions (Adapted from the publication of Kapil and Kapoor, 2005. With permission)

sensitive isolates does not infect these leaves (Pan-Yi Lou et al., 1997). The tolerant isolates have cross tolerance to benomyl, thiobendazole and thiophanate methyl (Shi Zhi Qi et al., 2000). Gossan et al. (2001) found resistance to benomyl in *S. sclerotiorum*. Pan Yi Lou et al. (2000) observed high proportion of carbendazim resistance in *S. sclerotiorum* at many sites of rape growing areas. The resistance and sensitive isolates are successfully detected by PCR amplification and that restric-

tion site (CGCG) at codons 197 and 198 (GACAG- > GACGCG) in MBCHR isolate (Li Hong Xia et al., 2002). Carbendazim resistance in *Sclerotinia* is due to single point mutation at amino acid 198 leading to substitution of glutamine for alamine in the pathogen (Li Hong Xia et al., 2003). Rapid identification of carbendazim resistance strains of *S. sclerotiorum* with in 6 h have been made possible using allele specific oligonucleotide (ASO) PCR technique (Li Hong Xia et al., 2004).

Chapter 20
Sclerotinia as Mycoherbicide

The terms "mycoherbicide" or "bioherbicide" or "biological herbicide" imply that the target weed is killed. All mycoherbicides do not necessarily kill, but have different effects on the target weed. Recent research has shown that like any other biological herbicide, *Sclerotinia* in the soil can have detrimental effects on seed germination and root growth without infecting and resulting in disease symptoms on the target weed (Boyetchko, 1996; Daniel et al., 1973). The idea of using plant pathogen for control of weeds was reported before the turn of the century, but it is only in the last three decades that it has received increasing interest (Charudattan, 1991; Freeman et al., 1978; Tebeest, 1996; Templeton, 1982; Watson, 1991; Wilson, 1969). More than 23 exotic plant pathogens have been investigated classical biological control of weeds, more than 67 weeds have been targeted using atleast 107 fungal texa as mycoherbicide agents (Mortensen and Hogue, 1995). However, *Sclerotinia* is effective as mycoherbicide on more than ten weed hosts (Table 20.1).

The potential of *S. sclerotiorum* as mycoherbicide against *Circium arvense*, *Colsium arvense* and *Cardecus mutans* in pastures has been discussed (Bourdot et al., 1996). In a field experiment conducted in a sheep grazed pasture in New Zealand, the effects of the fungus *S. sclerotiorum* on the long term dynamics of a population of *Cirsium arvense* have been determined by Bourdot et al. (2006). The pathogen is applied in mid-spring either once or thrice when the *C. arvense* shoots are vegetative rosettes, using a granular mycelium on boheat preparation that lodge on the host leaves, stems and in the leaf axils. The disease results in a temporary (17 months) reduction in population size through initial mortalities among treated shoots and results in reduction in root growth, adventitious roots, buds, subterranean shoots and aerial shoots population size. The *S. sclerotiorum* has potential as a mycoherbicide for *C. arvense* in sheep grazed pasture in New Zealand (Bourdot et al., 1995). Applications made during the spring and early summer months of October, November and December significantly reduces the ground cover of *C. arvense* by 67, 67 and 44 per cent, respectively. Reduction in ground cover is from 38 to 81 per cent (Hurrell et al., 2001).

Sclerotinia minor Jagger is a promising biocontrol agent for dandelion in turf grass. When a flowering dandelion population is treated with *S. minor*, flowering accelerates to the fruiting stage within four days. This developmental response is

G.S. Saharan, Naresh Mehta, *Sclerotinia Diseases of Crop Plants: Biology, Ecology and Disease Management*,

Table 20.1 Hosts on which *Sclerotinia* used as mycoherbicide

Host		Reference
Cirsium arvense L.	Canada thistle	Brosten and Sands, 1986; Harvey et al., 1994; Sands et al., 1990
C. vulgare L.	Scotch thistle	Bourdot and Harvey, 1994
Centaurea diffusa Lam.	Diffuse and spotted knapweeds	Mortensen and Hogue, 1995
C. maculosa Lam.	-do-	Sands et al., 1990
Taraxacum officinale L.	Dandelion	Riddle et al., 1991
Chrysanthemoides moniliferasub sp. *rotundata* (D.C.) Norl.	Bitou bush	Cother et al., 1996
Ranunculus acris L.	Giant buttercup	Harvey et al., 2001
Plantago major L.	Broad leaved plantain	Citola et al., 1991
Glechoma herderaceae L.	Ground ivy	Citola et al., 1991
Carduus nutans	Nodding thistle	Bourdot and Harvey, 1994
Senecio jacobaea	Ragwart	Bourdot and Harvey, 1994

four to five days earlier than in the control, untreated plants and is not observed in herbicide-treated plants. Seeds obtained from the fungal treated plants were smaller, lighter and their germination rate is reduced by 48.4 and 47.3 per cent for spring and fall applications, respectively. *S. minor* is not detected in dandelion seeds from the fungal-treated plants. In addition to effective control of mature (flowering) dandelions, seeds dispersed by dying plants have reduced germination and are not transferring *S. minor* off target (Abu Dieyeh et al., 2005). Sequential treatments of sub lethal rates (25 or 50 per cent of the recommended field rate) and *S. minor* (60 g/m^2) can interact positively to increase damage to common dandelion weed (Schnick et al., 2002). A mycoherbicide based on *S. sclerotiorum* has shown promise for the control of *Ranunculus* sub. sp *acris* (Giant buttercup) in New Zealand dairy pastures. Farm fertilizer management practices and moisture levels are likely to be important variables affecting the on farm efficacy of *S. sclerotiorum* used as a mycoherbicide for controlling giant buttercup (Pottinger et al., 2004). Mycelium on wheat formulation of fungus @ 500 kg/ha broadcasted in the infested pasture causes mortality of the giant buttercup plants upto 63 per cent (Verkaaik et al., 2004). However, Harvey et al. (2001) obtained 30–50 per cent reduction in the cover of giant buttercup when *S. sclerotiorum* is applied through broadcast treatment as dry kibbled wheat formulations. Crop disease risk after using *S. sclerotiorum* for weed control in pasture is defined as ratio of added to natural inoculum. Taking 1.0 to be a risk averse value for this ratio perimeter safety zone and 50 m wide are predicted for dairy and sheep grazed pastures (Bourdot et al., 2001). *S. sclerotiorum* has been found to infect *Chrysanthemoides monilifera* (bitoubush) at a number of sites along coastline of eastern Australia by Cother et al. (1996). In New Zealand, a strain of *S. sclerotiorum* isolated from Californian thistle has been found to be virulent also on scotch thistle (*C. vulgare*), nodding thistle (*Carduus nutans*) and ragwart (*Senecio jacobaea*) when applied as a mycelium on wheat formulation to the foliage of these weeds under green house conditions. Under field conditions,

this formation of mycoherbicide when applied to the new spring foliage of California thistle in sheep pastures, the weed is controlled to a high level both in the season of application and during the following growing season. Debilitation of root system of the thistle occurs through reduction in photosynthetic capacity, through death of treated shoots and by invasion and rotting of roots by the pathogen. The strain of the fungus used in the field does not infect either grasses or clover, therefore, it is an important and potential mycoherbicide for use in pastures to control weeds (Bourdot and Harvey, 1994). The integration of two or more methods in a weed control strategy may produce a positive interaction. For the control of common dandelion (*Teraxacum officinale*) in Canada, when *S. minor* prepared as a granular treatment of fungal colonized barley grits (20, 40 or 60 g m^{-2} rate) is applied in sequential treatments of sublethal rates of 2, 4–D (25 or 50 per cent of the recommended field rate) interact positively to increase the damage. This synergistic interaction may reduce the rate of either component required for adequate levels of control possibly decreasing the cost or volume of use of mycoherbicides in traditional weed control strategies (Schnick et al., 2002).

20.1 Resistance to Mycoherbicide

An anatomical study of the crown of giant buttercup (*Ranunculus acris*) and histopathological studies of infection of the crown by *S. sclerotiorum* have been carried out to assess the basis of crown resistance to mycoherbicide. Resistance is largely related to morphological features of the crown. Resistant crowns become thick at peripheral cortex, show deposition of lignified material at the margin of infected tissues, a response to wounding and the resistance of the crown's dense network of vascular tissues. These non- specific defense mechanisms limit infection within the crown of *R. acris* and enables recovery of the plant by regeneration from the crown buds (Green et al., 1998).

20.2 Formulations of Mycoherbicide

In general grain culture method has been used to formulate *Sclerotinia* mycoherbicide. Krietlow (1953) used 2:1 wheat: oat mixture for production of inoculum of *S. trifoliorum*. Riddle et al. (1991) used heat killed seed of perennial ryegrass colonized by isolates of *S. sclerotiorum* and *S. minor* to reduce the establishment of Dandelion by upto 85.5 per cent in field trials. Citola et al. (1991) obtained high rates of infection of *Plantgo major* L. (Broad leaved plantago), *Glechoma herderaceae* L. (Ground ivy) and Dandelion using coarsely ground barley seeds infected with *S. minor*. In Canada for the control of common dandelion (*Taraxacum officinale*) with *S. minor*, Schnick et al. (2002) have used a granular treatment of fungal colonized barley grits. Wheat kernels have been used as a solid carrier of the

fungus *S. sclerotiorum* for the control of *C. arvense* L. (Brosten and Sands, 1986). Bourdot et al. (1993, 1995) formulated *S. sclerotiorum* as mycelial infected granules kibbled (cracked) wheat for control of *C. arvense* in pastures. Field application of the granular for mutation results in deaths of a high proportion of *C. arvense* shoots in the year of application and plants have 60–70 per cent reduction in root biomass by the autumn following treatment (Bourdot et al., 1993, 1995). The application rate of the formulation is 50 g/m^2. Harvey et al. (1994) attempted to enhance the virulence of the kibbled wheat formulation of *S. sclerotiorum* by the addition of vegetable oil and the pathogen produced phytotoxin oxalic acid to the substrates. In field trials, Bourdot and Harvey (1994) obtained high level of weed control in New Zealand pastures when *S. sclerotiorum* is applied as a mycelium on wheat. *S. sclerotiorum* applied as a dry kibbled wheat formulation through broadcast in dairy pastures of New Zealand results in 30–40 per cent control of buttercup (Harvey et al., 2001). Water miscible formulations applied as slurry is less dependent upon leaf wetness than the dry granule formulations (Hurrell et al., 2001).

20.3 Constraints in the Development of Mycoherbicides

Several constraints in the development of commercial mycoherbicides need to be overcome. Mycoherbicide need to be fast acting, predictable, easy to use, environmentally safe and provide a level of weed control comparable to chemical herbicides before they will have general acceptance from industry and users. The use of plant pathogens as mycoherbicides is not without risks that need to be seriously and thoroughly assessed. Their safety, persistent survival and potential for genetic change are major concerns to scientist and regulatory bodies. Constraints to the development of commercial mycoherbicides can be categories as follows (Mortensen and Hogue, 1995).

20.3.1 Biological Constraints

Biological constraints include host variability and host range. Extreme host specificity of a mycoherbicide agent is an advantage where a weed is closely related to the crop in which it is to be controlled, however, where a number of weeds occur in a crop situation, host specificity is a disadvantage and other means of control are necessary. Factors like the morphology of the target weed can influence the level of control achieved. Generally grassy weeds (monocotyledons) are more difficult to control with foliar mycoherbicides because their growing points are well protected from infection by foliar pathogens. With most weeds, plant death is more likely to occur if pathogens infect stems at or below the cotyledonary node. Regeneration through regrowth from roots or rhizome buds is a major obstacle to achieve long term control of perennial weeds with foliar mycoherbicides like *Sclerotinia* (Smith, 1991).

20.3.2 Environmental Constraints

Suboptimal temperature and moisture are probably the most important environmental constraints for the efficacy of mycoherbicides. Moisture and dew period duration are main limiting factors to disease initiation and development. Appropriate timing of inoculum application to take advantage of humidity, dew periods and irrigation in the field can address this limitation. Formulations of foliar mycoherbicides with water retaining materials are considered a promising approach to make pathogen less dependent on available water initial infection (Charudattan, 1991; Templeton, 1982).

20.3.3 Technological Constraints

Mass production of viable, infective and genetically stable propagules like spores, mycelial fragments or pellets and sclerotia of a plant pathogen is a major requirement in the development of a mycoherbicide. Formulation of a mycoherbicide agent is one of the most challenging technological constraints to the development of reliable and efficacious mycoherbicide. One of the goals in formulating mycoherbicides is to keep the propagules viable and infective for a reasonable length of time preferably more than a year. In addition, a mycoherbicide should be formulated for easy delivery into agricultural systems, to enhance its effectiveness and to ensure consistency in activity. Adjuvant can be added to improve the adhesion and distribution of propagules in the host surface, enhance spore germination, germ-tube growth and appressorium formation. Ideally, formulations should be fully compatible with conventional application methods such as a boom sprayer or granular applicator (Boyette et al., 1996).

20.3.4 Commercial Limitations

The market for a mycoherbicide that target only one weed is quite restricted unless the product is active on an economically important weed that escapes control with traditional methods. The small niche markets of most potential mycoherbicides to date deters industry from getting involved due to limited opportunities to recover the cost of registration and large scale production (Charudattan, 1991).

Chapter 21
Phytotoxin, Phytoalexin, Fungal Viruses, Hypovirulence, Volatile Compounds of *Sclerotinia*

21.1 Phytotoxin Production and Phytoalexin Elicitation by *Sclerotinia*

S. sclerotiorum produces a somewhat selective phytotoxin "sclerin" which is phytotoxic to three cruciferous species (*Brassica napus*, *B. juncea* and *Sinapis alba*) susceptible to *Sclerotinia* stem rot disease, causing severe necrosis and chlorosis, but not to a resistant species (*Erucastrum gallicum*). Oleic acid, the major fatty acid isolated from sclerotia of *S. sclerotiorum*, is responsible for the toxic activity of extracts of sclerotia to brine shrimp larvae (*Artemia salina*). Phytoalexin elicitation in the leaves of *E. gallicum* results in the isolation of three known phytoalexins: indole-3-acetonitrile, arvelexin and 1-methoxyspirobrassinin. Considering that the resistance of *E. gallicum* to *S. sclerotiorum* is potentially transferable to *B. rapa*, a susceptible rape species and that arvelexin and 1-methoxyspirobrassinin are not produced by *B. rapa*. These phytoalexins may become useful markers for resistance against *S. sclerotiorum* (Pedras and Ahiahonu, 2004). In soybean, Glyphosate resistant lines S20-B9 and P 93 B01 produces more phytoalexins than Glyphosate susceptible S19-20 and P 9281 (Nelson et al., 2002a).

21.2 Fungal Viruses and Hypovirulence of *Sclerotinia*

Transmissible hypovirulence in *S. minor* has been observed by Melzer and Boland (1996) in culture and on lettuce tissue. Hypovirulence in fungal plant pathogens refers to the reduced ability of selected isolates within a population of a pathogen to infect, colonize, kill and (or) reproduce on susceptible host tissues and is often associated with fungal viruses and associated double-stranded RNA elements. It has been reported to occur in numerous fungal plant pathogens, including *Sclerotinia sclerotiorum*, *S. minor* and the disparate species *S. homoeocarpa*. In these fungi, hypovirulence has been associated with the presence of several fungal viruses, including one species of the genus *Mitovirus*, another species possibly belonging to the genus *Hypovirus*, and a satellite RNA. *Sclerotinia* spp. is primarily

G.S. Saharan, Naresh Mehta, *Sclerotinia Diseases of Crop Plants: Biology, Ecology and Disease Management*,

clonal in their life strategies with varying degrees of diversity manifested as vegetative compatibility groups within naturally occurring populations. Vegetative compatibility groups can reduce the frequency of transmission of fungal viruses between isolates that are not compatible. Agricultural populations of *S. sclerotiorum* typically consist of numerous clones, although several clones often represent the majority of a population within individual fields. In contrast, populations of *S. minor* and *S. homoeocarpa* are characterized by relatively few clones and may represent more promising pathogens for hypovirulence as a biological control strategy. Biological control has been demonstrated through applications of hypovirulent isolates to diseased plant tissues in controlled and field environments. In *S. minor*, disease severity is suppressed by more than 50 per cent and the number of sclerotia produced on treated diseased tissues is reduced by up to 90 per cent. These sclerotia are hypovirulent and contained double-stranded RNA characteristic of the hypovirulent isolate. In *S. homoeocarpa*, biological control efficacies of up to 90 and 80 per cent have been achieved in controlled and field environments, respectively and are comparable with treatment with a fungicide. Single applications of the hypovirulent isolate Sh12B, containing a strain of the species *Ophiostoma mitovirus* 3a (OMV3a) previously described from *Ophiostoma novo-ulmi* in Europe are as effective as up to four applications of fungicide and treatment efficacy persists into the following year. Collectively, studies of fungal viruses and hypovirulence in *Sclerotinia* spp. can increase our understanding of molecular mechanisms influencing the expression of virulence in these plant pathogens and expand the potential of fungal viruses as a unique mechanism of action for biological control (Boland, 2004). Interspecific transmission of double–stranded RNA and associated hypovirulent phenotype fungal plant pathogens (*S. sclerotiorum* to *S. minor*) by hyphal anastomosis have been obtained by Melzer et al. (2002). Association of ds RNA hypovirulence in isolates of *S. sclerotiorum* reduces or delay production of oxalic acid or both, incomparison with virulent isolates (Zhou and Boland, 1999). However, hypovirulence and its associated traits in *S. sclerotiorum* are not inherited in a Mendelian fashion (Jiang Dao Hong et al., 2000).

21.3 Volatile Compounds Emitted by Sclerotia of *Sclerotinia*

Volatile compounds emitted by sclerotia of *Sclerotinia minor*, *Sclerotinia sclerotiorum* and *Sclerotium rolfsii* have been identified by solid phase micro extraction followed by gas chromatography and mass spectrometry. Both *S. minor* and *S. sclerotiorum* emits 2-methylenebornane and 2-methylisoborneol. In addition, *S. minor* emits mesityl oxide, gamma butyrolactone, cis-and trans-linalool oxide, linalool and trans-nerolidol. *S. sclerotiorum* emits 2-methyl-2-bornene, 1-methylcamphene and a diterpene with a molecular weight of 272. *Sclerotium rolfsii* does not emit any of these compounds but did emit delta cadinene and cis-calamenene (Fravel et al., 2002).

21.4 Sporigermin from Sclerotia of *Sclerotinia*

A compound that specifically stimulates germination of macroconidia of *Sporidesmium sclerotivorum* [*Teratosperma sclerotivora*] has been isolated from the melanized outer layer of the sclerotia of *S. minor*. The compound is a small hydrophobic molecule that is able to pass through a 3,000 Da filter and is heat-labile in water. Three fractions are resolved using reversed-phase HPLC. The component with presumed biological activity in soil is sparingly soluble in water and has been named 'Sporigermin'. This is stated to be the first report describing the isolation of a compound from a host fungus that acts as a specific signal to stimulate germination of a mycoparasitic fungus (Mischke et al., 1995).

21.5 *Sclerotinia* Diseases as Health Hazards Problem

Sclerotia may be economically important because of their toxicity. Rats fed with 2 to 8 per cent sclerotia from sunflower head rots in the rations show delay of assification, decrease in maternal weight and lower food consumption (Ruddick and Harwig, 1975). Properly cleaned sunflower seed, however, is unlikely to contain concentrations of sclerotia high enough to create a health hazard.

Celery affected by pink rot disease (*S. sclerotiorum*) commonly causes dermatitis in workers who handle it (Scheel et al., 1963). The skin irritation is caused by two photo toxic furocoumarins, 8-methoxypsoralen and 4, 5, 8-trimethylpsoralen which are produced specifically by celery infested with *S. sclerotiorum* (Perone et al., 1964).

When weanling Wistar rats of both sexes are fed diets containing 0, 1 and 5 per cent ground sclerotia of *S. sclerotiorum* derived from infected rapeseed (*B. napus*), then, in the 5 per cent group, weight reduces, feed wastage is greater and at termination more than half the rats are in poor body condition with alopecia and hyperkeratosis of the tail. These effects are probably nutritional and due to unpalatability of the diet. Serum glutamic pyruvic transaminase activity is significantly depressed by consumption of sclerotia (Morrall et al., 1978).

Chapter 22
Laboratory and Field Techniques

22.1 A Rapid Screening Technique for Resistance

A method to inoculate detached bean leaves with *S. sclerotiorum* without using a carbon-nitrogen source has been described by Leone and Tonneijck (1990). Leaves are incubated in plastic trays to maintain air humidity above 95 per cent. Spores of the pathogen inoculated in water alone are generally unable to cause infection. Additions of KH_2PO_4 (62.5 mM) or of mixtures of inorganic phosphate and glucose (1.1 or 5.5 mM) to the inoculum stimulate fungal infection. Also spore concentration (2×10^5 or 2×10^6 spore's ml^{-1}) influences the ability to produce lesions. The fungus concentration (2×10^6 spores ml^{-1}) is able to infect primary bean leaves when inoculated in an inorganic phosphate solution. Since the procedure is simple and does not require blooming plants, it can be rapidly adopted in germplasm screening and breeding programs.

Wu and Liu (1991) indicated the possibility of *in-vitro* selection of rape (*B. napus* L.) callus cultures resistant to oxalic acid. However, Callahan and Rowe (1991) suggested that oxalic acid is not the sole inhibitory factor, yet other unidentified macromolecular components share a code terminate role in the inhibitory effect of lucerne *S. trifoliorum* system.

22.2 Germplasm Screening and Evaluation

22.2.1 *Pea*

The ascospores of *S. sclerotiorum* normally infect susceptible plants only if a saprophytic food base is available in the infection court, typically provided during flowering by fallen petals lodged in the leaf axis. Detached fresh petals of pea in a fresh state are suitable for the purpose. Oat grains colonized with mycelium are put on detached petals placed near the base of seedlings. Inoculation is carried out on 20 days old seedlings of the susceptible cv. Lincoln. Autoclaved soil is used to grow seedlings in 9 cm diameter plastic pots. Inoculated plants are maintained in a

G.S. Saharan, Naresh Mehta, *Sclerotinia Diseases of Crop Plants: Biology, Ecology and Disease Management*,

humidity chamber for seven days and transferred to glasshouse benches. Alternatively, petals are colonized with ascospores by inverting mature apothecial discs directly over petals for 3–5 min, since infection through ascospores is a bit cumbersome, so colonized oat grains on petals can be an efficient technique for evaluating resistant cvs. against white rot (Kapoor et al., 1990a).

22.2.2 Cauliflower

The procedure involves inoculating fresh curd with a precolonized cruciferous petal in a drop of water and keeping it in a humid chamber at 25 ± 1°C to serve as an inoculum source. To screen a crop in the field, 1 × 1 mm pieces of the colonized curd is then placed in the centres of the curds of the crop. Humidity is maintained by irrigation before and after inoculation and by mist spraying immediately after inoculation (Kapoor et al., 1986).

22.2.3 Rapeseed-Mustard

Thompson and Kondra (1983) used two techniques. One technique uses ascospores produced by apothecia under growth room conditions. The ascospores are collected and stored on micro filters, then suspended in water for inoculation. Flowering plants are sprayed with the ascospore suspension and kept in high humidity conditions. Symptoms development is recorded after two to three weeks. The second technique involves inoculation of stem of flowering plants with *S. sclerotiorum* growing on sterile medium. Inoculum is affixed to stem of plants using adhesive tape and the length of lesion developed around the inoculum is measured after two to three weeks.

Similar techniques were developed by Pierre and his associates where sclerotia of the pathogen are obtained from diseased plant tissue or produced *in vitro*. Sclerotia are put in pots between two layers of perlite, kept outside. Pans are watered once in a week and covered with black plastic cover. Three months later, sclerotia are sorted, cleaned and put in pots filled with compost in a green house kept at 15–18°C. Stipes appear two to six weeks later or else. The induction takes place in the field. Sclerotia put in sealed bags of plastic etamine are placed 10 cm deep in the soil. Sclerotia must be entirely surrounded by the soil. Four months later, sclerotia are washed and sorted out and put in small closed crystallizers on a cotton wool bed in the light, at a humidity approaching saturation and at a temperature of approximately 20°C. Stipes develop two to three weeks later. As soon as stipes appear, sclerotia can be used directly in the field, or prepare ascospore inoculation for spray inoculation. Light is essential to obtain apothecia. Pots containing sclerotia with stipes are put in a greenhouse or in a long-day lit room sheltered from direct sunlight. Crystallzers can either be covered with a transparent lid, or be kept

in the open air, if the atmosphere has a high relative humidity. In the case of pots, sclerotia and their stipes are covered with small, transparent plastic beakers. Inoculum can be collected in two ways: In the first case, when discs of apothecia are formed, spores are collected over several weeks twice to three times per week. When the beaker lid is removed, a cloud of ascospores is released and gathered on a millipore filter with meshes inferior to 5 µm using rapid aspiration. The principle of the device is simple, a funnel shaped container with a filter at the bottom supported by a thin grid. Aspiration is carried out using a household vacuum cleaner. In the second case, mature apothecia are sampled in the morning and put in a Petri-dish. Spores are released by progressive drying.

For sclerotial soil inoculation, as soon as stipes appear, two small boxes containing five to ten sclerotia are put at soil level for each 15 m^2 plot at the beginning of rapeseed flowering. Symptoms of stem rot appear about a month later. It is recommended to cover the trial with a canvas sheet for a month to maintain temperature and humidity.

For ascospores spray inoculation. Ascospores collected on filters or in Petri-dishes are put into suspension by scrapping. Most often spores appear in the form of clusters which can be separated by strong manual shaking followed by a few seconds in an ultrasonic vat. The volume of spore suspension is adjusted to obtain 10^4–10^5 viable spores/ml. Ascospores inoculum spraying is usually carried out when rapeseed petals fall, in the first period of flowering. A complementary supply of petals, naturally dried or heated for 1 h at 100°C can be obtained. Scheibert-Bohm et al. (1981) used several inoculation methods viz., (1) infected kernels of oats and rice, (2) mycelium suspension and/ or cotton, (3) mycelium suspension, cotton and parafilm, (4) tooth prick and or tooth pick and cotton and (5) infected pieces of agar. Best results are obtained with infected oat kernel and infected agar pieces under long day and low temperature conditions. In infected oat kernels, a mixture of 1:1 kernels and vermiculate is used as inoculum. It is spread around the plants or over the planted seeds. Fifty grams of infected oat kernels and 50 g of vermiculate is used per plastic dish. The infected agar pieces of 5 mm size are pressed to the hypocotyls of the test plant at three leaf stage and wrapped with parafilm.

22.2.4 Sunflower

To prepare the inoculum, three 11 × 12 cm sheets of bathroom tissue (Charmin, plain, white consented) are placed in 100 × 15 mm Petri plates and 15 ml of a solution of 1 per cent yeast extract, 1 per cent casamino acids and 2 per cent dextrose is added to each plate. The tissue medium is sterilized then small plugs of agar with mycelium (from seven to ten days old PDA cultures) are placed around the periphery of the tissue. A dense mycelium covers and permeates the tissue after seven to ten days incubation at 20°C. The tissue and mycelium is cut into 35 × 35 mm pieces and inoculated on to sunflower (Nelson, 1985b).

A seedling test to evaluate virulence of *S. sclerotiorum* on sunflower was described by Nelson (1985a). Surface sterilized seeds of test cvs. are placed in sterile, wet, seed germination paper rolls (Anchor paper) and incubated seven days at 20°C. The seedlings are then inoculated by placing mycelium + agar plugs from PDA culture of the pathogen on to the bases of the stems. Inoculated seedlings are incubated for 72 h at 20°C. Virulence is measured as the length of decayed stem tissue.

A sunflower head inoculation techniques developed by Vear and Guillaumin (1977) consisted of placing agar containing mycelium on the sterile surface of the capitulum without injury. There is a significant correlation between results in a growth chamber at 18°C and in the field. Fungal spread is inversely related to the maximum temperature on the day after inoculation. Resistance to basel stem attack determined by inoculation with sclerotia at soil level is not always related to resistance to capitulum attack. Spraying the heads at the beginning of flowering with 5 cm^3 of a suspension of ascospores (5,000 ascospores/ml) and covering the heads with brown paper bags immediately after inoculation is recommended by Becelaere and Miller (2004) for identifying resistance. No application of additional water is required.

While comparing three methods to assess resistance in sunflower to basel stem rot caused by *S. sclerotiorum* and *S. minor*. Sedun and Brown (1989) found linear rate of lesion development as to be a good measure of relative resistance under laboratory and field conditions. Inoculum is produced by growing isolates on moist autoclaved wheat grain (170 g wheat and 150 ml distilled water in a 500 ml conical flask) for ten days at 25°C in darkness. Sunflower seedlings are grown in 10 cm diameter plastic pots containing a mixture of peat, vermiculite and sand in a controlled environment cabinet with 16 h days (450 µ E. m^{-1} S^{-1}) at 24°C and 8 h nights at 18°C. Pots are watered twice daily and fertilized once every week with a complete fertilizer. At 30–35 days after sowing, 15 g of inoculum consisting of wheat grain and mycelium is inserted into the outer edge of the soil about 5 cm in contact with the plants roots. The development of lesion on the stem is measured daily from the first appearance of the lesion above the soil line until the lesion front is no longer discernible. The rate of lesion expansion for each treatment is calculated as the slope resulting from the linear regression of lesion length on time (days). Daily observations are taken on the development of wilt symptoms on leaves and stems. Under field conditions, a randomized block design is used with each treatment replicated three times. Plants are grown in four-row plots, 4 m in length, with an inter row spacing of 0.75 m. Shortly after emergence, seedlings are thinned to one plant every 0.25 m of each row. At the early bud stage of plant growth, the middle two rows of each plot are inoculated with 50 ml (25 g) of inoculum inserted into a 3 cm diameter × 10 cm hole located 10 cm from the base of each stem. The number of plants that showed symptoms of wilt and basal stem lesions is recorded daily for 41 days after inoculation. The use of the rate of basal stem lesion expansion to rank sunflower lines for resistance to *Sclerotinia* provides very consistent results in both field and laboratory studies.

A simple, rapid, effective and reproducible sunflower seedling resistance technique through the pathogens toxic metabolites has been developed by Huang and

Dorrell (1978). The strain of *S. sclerotiorum* (Ss3) isolated from infected sunflower roots is purified by single hyphal tip isolation and maintained on PDA. Culture filtrates are prepared by growing the pathogen in a liquid-salts-yeast extract medium (pH 5.8) supplemented with 73.7 mM D-glucose and 56.0 mM sodium succinate (Maxwell and Lumsden, 1970). Three discs (12 mm diameter) from seven to ten days old cultures grown on PDA plates are transferred to Erlenmeyer flasks containing 250 ml of the liquid medium. The culture is incubated at room temperature for two weeks. Culture solutions are filtered through Whatman No.1 paper and then autoclaved for 15 min at 20°C. The roots of test seedlings with two pairs of true leaves or approximately three weeks old are washed free of soil in tap water. Ten seedlings are placed in a 50 ml vial containing 45 ml of the culture filtrate. At least 50 seedlings of each germplasm line are tested with the culture filtrate in three replications. Controls consist of ten seedlings of each line in a vial containing the liquid-salts-yeast extract medium. The seedlings are incubated at room temperature for 24 h with 12 h of fluorescent light. Wilting is rated on a 1–4 scale. A wilt index (W) is calculated for each line using the formula:

$$W = E\,\frac{(nw)}{T}$$

(n = number of seedlings, w = wilt rating, T = total number of seedlings)

Putting seed-carrying mycelium near the collar of the root and covering it with wet soil, or placing agar containing mycelium on the surface of the capitulum, or any modification of the mycelial inoculation method, has been used as an inoculation method for evaluation of resistance (Kolte et al., 1976; Shopov, 1976; Vear and Guillaumin, 1977). Under natural conditions, the wilt phase of the disease appears at the budding stage and the mycelial inoculation technique is expensive and laborious. Instead, the use of the culture filtrate of *S. sclerotiorum* is suggested, which can provide a reproducible, rapid and simple technique for screening sunflower seedlings and could be a useful adjunct to field nurseries in evaluation of the wilt phase of the disease (Huang and Dorrell, 1978; Noyes and Hancock, 1981).

22.2.5 Field Peas

Inoculum is prepared by culturing a surface sterilized field sclerotium on PDA for three days at 25°C ± 2°C. A mycelial plug is cut at the growing margin of a colony and transferred to a 125 ml Erlenmeyer flask containing 25 ml of glucose nitrate medium (Vega and Le Tourneau, 1974). The fungus is grown in shake culture for five days. The liquid culture is aseptically blended for 30 s in a Warring blender and added to 1,000 ml mason jars containing 85 g of autoclaved oat kernels and 150 ml distilled water. During the following five days incubation period, oat cultures are shaken once a day by hand to ensure even growth of the fungus. Prior to inoculation, infested oat kernels are separated and mixed thoroughly by hand. They are

allowed to incubate in a closed container 24h before inoculation. Pea seeds are surface sterilized in 5.25 per cent sodium hypochlorite for 10 min, rinsed with water and planted in autoclaved wooden flats in a greenhouse. Flats contain an artificial potting mix (pH 5.5 to 6.0) consisting of equal parts by volume of sand, peat and perlite supplemented with lime, osmocote fertilizer (14-14-14) and micronutrients. Metal halide lamps are used to provide a 15 h photoperiod centered around the natural day length. Day and night temperature is 21°C and 16°C, respectively. Ten days after planting, seedlings are placed in mist chambers covered with polyethylene. Seedlings are predisposed by misting once every 10 min for 24 h. Seedlings are inoculated by placing one infested oat kernel in contact with the base of each stem. Inoculated plants are misted once every 10 min for 6 h a day and kept at 18°C temperature (Blanchette and Auld, 1978).

22.2.6 Lettuce

The pathogen isolate obtained from field infected lettuce is maintained by periodic transfers on Difco potato dextrose agar (PDA) at 20–22°C. Infected rye (*Secale cereale* L) seeds are used as the source of inoculum. About 10 g of rye seeds are placed in 500 ml flasks with 20 ml of distilled water and autoclaved. After cooling, each flask receives three discs (6 mm diameter) from the margins of advancing fungal colonies on PDA plates. All flasks are incubated for five to seven days at 25°C before use. Seeds of lettuce germplasm are planted in steamed, coarse sand. Seedlings are transplanted two weeks later into 10 cm diameter plastic pots (four seedlings per pot) filled with steam treated soil mix. The plants are grown in a greenhouse at 20–24°C and fertilized weekly with a complete nutrient solution. Then, three to four weeks after transplanting, each seedling is inoculated with three rye seeds colonized by *Sclerotinia*. The inoculum is placed in contact with the taproot about 1–2 cm below the soil surface. Seedlings of each selection that receive three autoclaved and non-colonized rye seeds serve as check plants. All plants are maintained in the same greenhouse and watered as needed, once or twice daily. The number of infected plants is recorded 7, 14 and 21 days after inoculation. All surviving plants from each accession are reinoculated three weeks after the first inoculation (Abawi et al., 1980).

22.2.7 Beans

In a limited-term inoculation method to screen bean plants for partial resistance to white mould, Hunter et al. (1981) used small (4 × 8 mm) pieces of colonized celery petiole as inoculum. The petiole pieces are autoclaved and placed cut side down on a plate of PDA colonized by a rapidly growing culture of the fungus without sclerotia. After incubation for 24 h at 22°C, the celery pieces are placed on a second or

third internodes of four to five weeks old bean plants and a piece of wet absorbent cotton is wrapped around the stem to hold the celery pieces in place. Inoculated plants are incubated in a growth chamber for 48 h with 12 h of fluorescent light at 21°C and about 90 per cent relative humidity. The cotton and celery are then removed. The plants are kept in a greenhouse for three days and then rated for disease.

22.2.8 Soybean

Three or four soybean plants representing cvs. from groups II–IV are grown in 25 cm clay pots in the greenhouse with supplemented lighting. Plants are hardened off in a growth chamber with a 14 h photoperiod for one week before inoculation. The *S. sclerotiorum* isolate used in this study is recovered from sclerotia formed on naturally infected soybean plants and maintained on potato dextrose agar. Either ascospores or autoclaved carrot or celery pieces colonized by the fungus are used to inoculate soybean plants. In some tests, inoculated plants are placed in a mist chamber at 20–25°C with a 12 h photoperiod and relative humidity maintained near 100 per cent by a mist system that sprays plants for 15 min every hour for the first three days and 15 min every 2 h for the remaining incubation period. In other tests, plants are placed in premoistened polyethylene bags in a growth chamber at 21°C with a 12 h photoperiod. Plants are removed each day, sprayed with distilled water until runoff and rebagged. After specified incubation periods in either the mist chamber or the growth chamber, plants are rated for disease severity according to a scale of 0–5.

22.2.8.1 Ascospore Inoculations

Apothecia are produced using the technique described by Kohn (1979a) except sclerotia are incubated in sterile preparation dishes containing vermiculite rather than glass wool. Ascospores are collected and the concentration adjusted to $1–5 \times 10^5$ spores per milliliter and the suspension is atomized onto each plant. Plants in full bloom are inoculated and placed in the mist chamber and rated for disease development after 3, 7 and 14 days.

22.2.8.2 Colonized-Carrot Inoculation

Five millimeter mycelial agar plugs containing hyphal tips from the advancing margins of three to four days old colonies are transferred to autoclaved carrot root pieces in 9 cm glass Petri dishes. After incubation at 21°C for 24 h, a 5 mm plug of the colonized carrot piece is placed on the center leaflet of the oldest trifoliate leaf of four plants in the V4–V5 growth stage (four weeks old) from each of test cvs.

Plants are placed in the mist chamber and rated for disease development after 7 and 14 days. Controls are inoculated with uncolonized autoclaved carrot pieces.

22.2.8.3 Limited-Term Inoculation (LTI)

Celery petiole pieces (4 × 8 mm) that had been colonized by the fungus for 24 h are placed in either the second or third node of four weeks old soybean plants (V4–V5). The stems are then wrapped with a piece of moistened cotton, bagged and placed in the growth chamber. The inoculum and cotton are removed after 24 h and the plants are wetted, rebagged and placed back in the growth chamber and rated for disease development after seven days.

Limited-term inoculation is the only successful inoculation method of the three tests in distinguishing differences in disease susceptibility among the soybean cvs. evaluated. Results of ascospore inoculation method are variable. Disease severity ratings of plants inoculated by the LTI method can be affected by the age of the tissue and the light intensity under which plants are grown. LTI inoculation is a feasible method for evaluating stem rot because the disease severity ratings obtained by this method parallel observation made on field infected plants (Cline and Jacobsen, 1983).

Chun et al. (1987) developed another method to assess resistance in soybean against the stem rot pathogen under laboratory and field conditions. In this method, pathogen isolate A from navy bean and isolate G from soybean are maintained on millet seed agar. Millet seed is ground in a Wiley Mill to pass through a sieve with 0.85 mm openings (20 mesh). Twenty grams of ground seed is mixed per litre of 2 per cent agar and autoclaved, then 20 ml is poured into 9 cm diameter Petri dishes. The agar is inoculated in the center of the plate and cultures are grown at 23 ± 1°C for 3–14 (usually 5) days. The inoculum consists of 5 mm diameter discs cut with a cork borer from two concentric circles 1–1.5 cm from the edge of the culture. In cultures older than five days, sclerotial initials begin to form.

Soybean plants are raised through seeds germinated in moist vermiculite for two days. Uniform seedlings are transplanted into 11 cm diameter × 14 cm plastic pots of 946 cm^3 capacity containing a potting mix, with three to seven (usually three) plants per pot. Plants are grown for three to seven weeks in a greenhouse at various times of the year. The temperature in the greenhouse ranges from 20°C to 35°C and day lengths extend to 12 h in the winter by fluorescent lamps. Plants are cut off at ground level, put in polyethylene bags and brought into the laboratory, where leaves and growing tips are excised before inoculation.

For inoculation, excised tips are placed on 500 cm^3 of moist silica sand (1 cm deep) or 100 cm^3 of vermiculite (2 cm deep) in plastic trays 26 × 18 × 6 cm. The trays are first lined with a single layer of plastic film, two or three layers of which are also used to cover the trays to retain moisture. The sand is moistened with 200 ml of distilled water and the vermiculite with 500 ml distilled water, 12–16 stems are arranged parallel to the short axis of each tray. Stems are inoculated by applying discs of inoculum at various sites on the stems. Inoculum is applied either

to the axil of the first trifoliate leaf or on the cut apex of the stem. To aid in adhesion, each disc is dipped in 0.3 per cent water agar before being applied to the stem. Trays with inoculated stems are incubated on a laboratory bench for five to seven days at about 25 ± 3°C or 21 ± 1°C. Lesion lengths are measured as the distance from the site of inoculation to the farthest macroscopically visible extent of the lesion. Mean lesion lengths in each replicate are calculated as the sum of individual lesion lengths divided by the total number of inoculated plants (whether infected or not). Field screening is done in artificially infested soil with sclerotia of the pathogen.

22.2.8.4 A Green House Spray and Drop Mycelium Inoculation Method

In a spray mycelium inoculation method, *S. sclerotiorum* mycelia are cultured in liquid potato dextrose broth and homogenized before spraying on the soybean leaves. In "drop-mycelium" methods, a drop of homogenized mycelium suspension is dropped on the tips of main stems. Inoculated plants are incubated in a greenhouse chamber with 60–80 per cent relative humidity. Plant mortality and Area Under Wilt Progress Curve (AUWPC) are used to measure disease severity daily from 3 to 14 days after inoculation (DAI). The spray mycelium method and the drop mycelium method are compared with the cut-petiole method in the greenhouse. Significant differences in disease ratings of plant mortality and AUWPC to *Sclerotinia* stem rot are found among tested genotypes. The results obtained with the spray-mycelium and drop-mycelium inoculation methods are significantly ($R > 0.73$, $P < 0.01$) correlated with the results obtained with the cut-petiole inoculation method for both of the plant mortality and AUWPC. Compared with the cut-petiole method, the spray-mycelium and the drop-mycelium methods uses less inoculation time and are less expensive in terms of materials. Both of these new methods are low cost, efficient, and reliable and can be valuable for large-scale evaluation of germplasm and breeding lines for resistance to *Sclerotinia* stem rot in a greenhouse or other similar facilities (Chen and Wang, 2005).

22.2.8.5 A Field Inoculation Technique to Assess Partial Resistance in Soybean to *Sclerotinia sclerotiorum*

The barley kernel inoculation technique involves stem inoculation using an infected barley kernel inserted into a wound made in the soybean stem. The damage on soybean plants is measured by the length of the lesion on the inoculated stem and expressed as inoculation severity index (ISI). This may be of value to soybean breeders trying to develop soybean lines resistant to *Sclerotinia* stem rot because of the difficulty in obtaining consistent and reliable DSI scores across locations and over years. Soybean cultivars evaluated for two years using the barley kernel inoculation technique and compared with other controlled environment and natural infection revealed that the correlation between the DSI from some locations and years

and the ISI was negative. The significant positive correlations obtained using soybean lines that were not previously selected for DSI suggest the potential of this technique for pre-screening the breeding material for resistance to *Sclerotinia* stem rot prior to confirmation with natural infection levels as measured by DSI (Auclair et al., 2004b). According to Chaves et al. (1996a), the particles of dried mycelium of *S. sclerotiorum* are infective when deposited near the root collar of young plants. This method has been used in Brazil to test commercial soybean varieties for resistance.

To evaluate resistance against *Sclerotinia* stem rot of soybean and dry bean, out of three methods viz., mycelial plug inoculation of cotyledons, cut stems and detached leaves tested, cut stem method is better and more precise for detecting interactions (Kull et al., 2003). Wegulo et al. (1998) suggested that determination of levels of soluble pigments in stems. Measurement of lesion length on stems discoloured by oxalic acid and detached leaf assay may be better than mycelial inoculation of stems or foliage in evaluating soybean cultivars for field resistance to *S. sclerotiorum*.

22.2.9 Forage Legumes

Sclerotia of *S. trifoliorum* produced in the laboratory are buried 1–2 cm deep in permanently soaked vermiculite at 15°C, 12/24 h of fluorescent light and RH > 80 per cent in order to differentiate apothecia. The ascospores are harvested by aspiration and collected on a membrane filter. Various types of apparatus are tested for their ability to maintain live apothecia and for spore catching. The aspiration method is found to be much more efficient than the previous method of cutting off apothecia and the best apparatus consists of small, individual compartments (truncated plastic bottles). The ascospores can be stored on the membrane filter at 5°C and low RH to preserve their germinating ability for a period of three months at least. This spore harvesting method can be used to prepare large quantities of *S. trifoliorum* inoculum for resistance testing of forage legumes all year round (Delclos and Raynal, 1995).

22.2.10 Alfalfa

Screening for resistance to *Sclerotinia trifoliorum* in alfalfa by inoculation of excised leaf tissue: Excised lucerne leaf tissues are inoculated with *S. trifoliorum* to determine whether leaf-inoculation techniques can be used to screen for heritable and effective resistance to the pathogen. Leaf tissues are placed on water agar in Petri plates, inoculated with mycelia of *S. trifoliorum* and incubated at 17°C. Host responses are evaluated according to the rate and extent of necrosis that develops during first week. Plants of varieties/lines/cultivar are screened for resistance by

successive inoculation of excised unifoliate leaves, first trifoliate leaflets and discs of tissue from later leaves. The five plants considered most resistant are intercrossed and progeny are compared with those of five plants selected for susceptibility, five plants selected at random and the parent cultivar. Progeny of resistant plants have significantly less severe disease than does progenies of susceptible and random plants and the parent cultivar. In field experiments with natural infection during two growing seasons, progeny of resistant plants have less severe disease and produced significantly higher yields of forage through the first or second harvests than progenies of susceptible and random plants and the parent cultivar. It is concluded that inoculation of excised leaf tissue is an effective and efficient way to screen for resistance to *S. trifoliorum* in lucerne (Pratt, 1996). The initial screening for resistance to *S. trifoliorum* in alfalfa seedlings may be accomplished by applying wounded unifoliate leaves and leaflets of trifoliate leaves directly to colonies on cornmeal or V8 juice agars (Pratt and Rowe, 1998).

22.3 Field Inoculation of *Sclerotinia*

Successful artificial field inoculations of red clover with *S. trifoliorum* can be obtained by scattering dried grain inoculum over the plants. The inoculum is usually used at the rate of 1–12 g per square foot (Graham and Hanson, 1953).

For faba bean, different organic materials colonized by the fungus, *S. sclerotiorum* have been used as energy rich inocula by Lithourgidis et al. (1989). These materials are colonized pieces of celery stem, faba bean petiole and carrot roots, blocks of fungal culture on PDA and pieces of sclerotia. The inocula are left attached to the stem for 48 h, then removed, after which the plants are assessed for disease incidence and severity. The results are most uniform when colonized pieces of carrot roots are used as inocula.

22.4 Separation of *Sclerotinia* sclerotia from Soil

Sclerotia of *Sclerotinia* spp. can be separated from artificially and naturally infested organic soils which involves wet-sieving of the soil and flotation of the sclerotia in 70 per cent glycerol. In the procedure, soil samples are air-dried, sifted on 2 mm wire mesh, blended in tap water in a Warring Blender and wet sieved through 2 mm (9 meshes) and 297 µm (48-mesh) sieves. Residue on the 2 mm sieves is discarded and the residue on the 297 µm sieve is transferred (after washing) to a centrifuge tube containing 70 per cent glycerol. After centrifugation for 3 min at 3,000 rpm, the sclerotia in the overlying liquid are removed, counted by using a stereoscopic microscope, surface sterilized and then plated on acidified potato dextrose agar to determine viability. The average percent recovery of sclerotia by this procedure is 93 per cent. A schematic diagram of the wet-sieving flotation procedure used for

the separation of sclerotia of *S. minor* from artificially or naturally infested organic soil is given in Fig. 22.4.1 (Abd-Elrazik and Lorbeer, 1980).

A wet sieving flotation technique for isolation of sclerotia from much soil has been described by Utkhede and Rahe (1979). Soil samples (20 g) are washed through two stacked sieves (0.595 mm openings over 0.210 mm openings) for at least 5 min and the residues on the 0.210 mm sieves are transferred to columns containing 2.5 M sucrose solution (1.330 sp, gr.) After 2 h, the soil fractions suspended in the upper portions of the columns are collected, washed with water on 0.210 mm sieves and examined with a dissecting microscope. Sclerotia are removed with forceps, surface sterilized in 0.25 per cent sodium hypochlorite for 2.5 min, washed in distilled water and cut in half. The two halves are placed on potato dextrose agar in Petri dishes and kept at room temperature (22–25°C) for two weeks to allow identification of the pathogen. Approximately 82 per cent of sclerotia can be recovered from naturally infested soils by this method.

Porter and Steele (1983) elutriated sclerotia of *S. sclerotiorum* causing blight of peanut from soil with a semiautomatic elutriator. Sclerotia are collected on 425 µm-mesh sieves during elutriation. The recovery of sclerotia from artificially infested soil following 3.0, 4.4, 6.75, 10.0, and 15.0 min of elutriation (64 ml of water per second and 231 cm^3 of air per second per unit) is 65, 83, 92, 94 and 97 per cent,

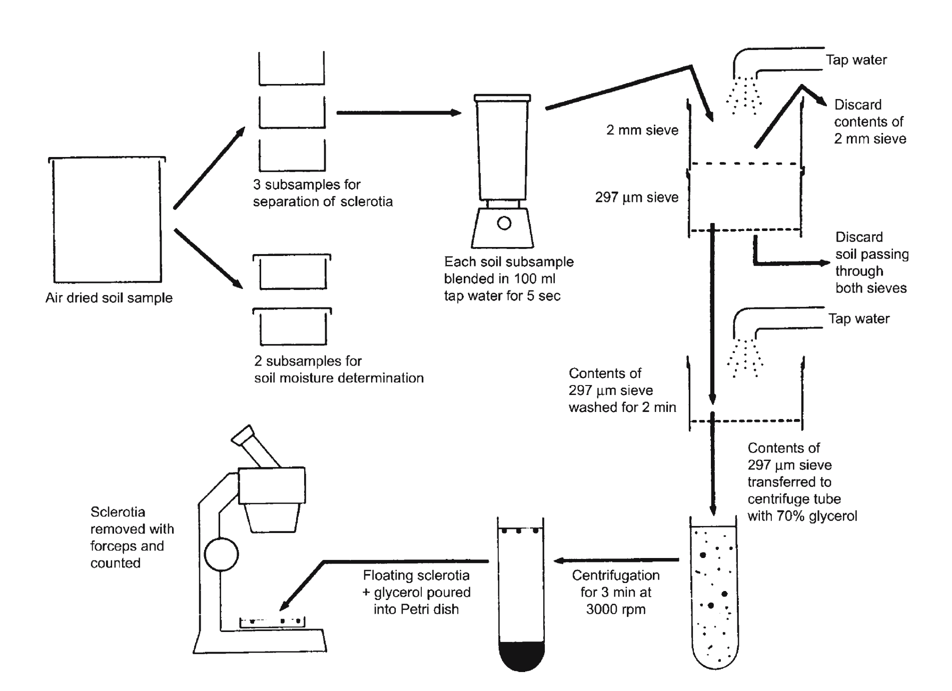


Fig. 22.4.1 Schematic diagram of the wet-sieving flotation procedure used for the separation of sclerotia of *Sclerotinia minor* from artificially or naturally infected organic soil (Adapted from the publication of Abd-Elrazik and Lorbeer, 1980. With permission)

respectively. Sclerotia can be found throughout the plough zone (20 cm depth) in fields with histories of *Sclerotinia* blight.

22.5 Apothecial Production

For apothecial production cultures, grown and maintained on potato dextrose agar are transferred to PDA in 9 cm Petri plates, incubated for three to four days at room temperature, then transferred with a 5 mm cork borer from the growing margin of the colony to 500 ml Erlenmeyer flasks containing autoclaved carrot discs and 25 ml distilled water. The flasks are incubated for four weeks without light at 15°C. Sclerotia are harvested, rinsed in distilled water and transferred to sterile preparation dishes containing glass wool saturated with distilled water. The sclerotia are then "cold conditioned" for four week at 0°C. The dishes are removed to a growth chamber set at 15°C, with fluorescent and incandescent light at approximately 21,520 lux and a 14 h photoperiod. Apothecial initials appear 4–12 week after introduction to the growth chamber (Kohn, 1979a).

According to Hawthorne (1973), for production of apothecia of *S. minor*, sclerotia are produced by growing the fungus on whole meal agar (50 g whole meal flour, 20 g agar, 1,000 ml distilled water) for four weeks at 18°C, then they are scraped from the agar surface and air dried for three to four days. The air-dried sclerotia are screened on an 18 mesh sieve, and those that pass through are discarded since stipes formed by these small sclerotia very rarely develops into apothecia. One hundred milligrams of the screened sclerotia are added to 9 cm Petri dishes containing about 20 ml tap water and the dishes incubated for six to eight weeks in the dark, in an incubator operating at 15°C for 8 h and 10°C for 16 h. At the end of this period large numbers of sclerotia have produced stipes, but these are not differentiated into apothecia because of the absence of light in the incubator. Mature apothecia are produced by illuminating the stipes for 8 h each day at 15–18°C for 14–21 days with two 40 W daylight fluorescent lamps held 90 cm above a single layer of Petri dishes.

For production of apothecia of *S. trifoliorum*, fungal isolates are grown on malt extract agar at 20°C temperature. Numerous sclerotia are produced on 10 per cent malt extract exposed to near UV on a 12 h dark, 12 h light cycle in two weeks time. The sclerotia are removed aseptically from the agar and placed on 1 per cent tap water agar, several spaced on a Petri dish. Apothecia begin to develop after about six weeks if the dishes are incubated at 10°C but somewhat better results are obtained if the sclerotia on the tap water agar are first placed in a deep freeze at 18°C for 48 h. Best results can be obtained by alternating a 16 h period at 2°C with 8 h at 8°C. In the dark, stipes only are produced but if white light is provided (12 h per day or 8 h per day in the case of the alternating temperature regime) during the incubation period, normal apothecia are formed. Light intensity (710 lux) is important to get good results with a white fluorescent tube at a distance of 30 cm (McGimpsey and Malone, 1979).

According to Mylehreest and Wheeler (1987), an isolate of *S. sclerotiorum* from oilseed rape cv. Jet Neuf is grown at 20°C in 250 ml Erlenmeyer flasks on autoclaved wheat grain (25 g wheat, plus 60 ml distilled water autoclaved at 20°C for 20 min). After three weeks, when sclerotia have formed and matured, the flasks are incubated at 4°C for four weeks. The sclerotia are then removed from adhering wheat grains and placed 1 cm deep in John Innes No.1 compost in round, plastic containers with lids. The dishes are kept at l0°C and the compost kept moist by spraying with distilled water periodically. When stipes are visible (six weeks), the dishes are placed about 35 cm beneath near UV light for a 14 h exposure per day at 22°C. Apothecia develop within five days and when the lids of the dishes are removed, discharge of ascospores in masse from apothecia (puffing) is clearly seen. By this method, apothecia with high fertility can be produced in about 15 weeks.

Both newly formed sclerotia and those treated with 20°C for three or six months produce apothecia in a relatively long time (ca. 120 days) after being placed under optimum conditions, but more apothecia are produced by the later. On the other hand, sclerotia treated with 20°C for three months and then with 0°C for another three months, produce apothecia very rapidly (ca. 50 days) under optimum conditions. There is no apothecial development at 0°C for three to six months (Terui and Harada, 1966).

Method described by Smith and Boland (1989) results in the reliable production and maintenance of stipes from sclerotia of *S. sclerotiorum* at 4°C. In this method, isolates of *S. sclerotiorum* are initially cultured on PDA medium. Five agar plugs (6 mm diameter) are cut from the actively growing colony margins and transferred to 125 ml Erlenmeyer flasks containing wheat seed and water (1:1 W/V) that has been autoclaved at 121°C for 20 min. Cultures are grown at 20–22°C for 14 days in the dark and then pretreated at 4°C for at least six weeks in the original flasks containing the wheat substrate and sclerotia. Some isolates are also grown on white bean seed and soybean seed mixed with water (1:1 W/V*)* and/or on PDA. Following pretreatment at 4°C, sclerotia are separated from the seeds, surface sterilized in 0.6 per cent sodium hypochlorite for 2 min, rinsed twice in sterile distilled water and incubated at 4°C in sealed plastic bags in the dark. Weekly observations for the development of stipes are made. After individual isolates have produced stipes at 4°C in the dark, the potential for stipes to produce apothecia after prolonged storage is evaluated by transferring sub samples of 10–20 sclerotia with stipes to an incubator at 16–20°C with a 14 h day length at 150 μE s^{-1} m^2 radiation supplied by fluorescent and incandescent bulbs. Sclerotia are placed in moist sterile silica sand in 9 cm Petri dishes sealed with Parafilm. Frequent observations are made for the development of apothecia. Sclerotia grown on wheat and/or PDA produce stipes after 4–40 weeks of incubation at a constant temperature of 4°C in the dark.

After carpogenic germination in individual isolates at 4°C, fertile apothecia are produced from stipes within 10–30 days by transferring sclerotia with stipes to 16–20°C in the light. Sclerotia with stipes can be maintained at 4°C in the dark for up to 18 months before being transferred to 16–20°C in the light and these sclerotia continue to produce fertile apothecia within 10–30 days after transfer. The feature of this method allows for a continued supply of germinated sclerotia with stipes, which are maintained at 4°C until required for the production of apothecia.

22.6 Ascospore Collection

The technique used for collection of ascospores consists of positioning directly above a mature apothecium, a millipore funnel with a 25 mm filter holder attached to a vacuum pump. A short breath of air exhaled over the apothecial disc induces ascospore discharge. The spores are discharged and captured on Whatman No.1 filter paper as often as once every 3 h from either apothecia which have emerged from sclerotia in the soil in the greenhouse or apothecia produced by sclerotia on agar plates in the laboratory. Ascospores are stored at 22°C on the filter in Petri-dishes. When washed from the filter paper into distilled water immediately, three months or seven months after collection, the ascospores germinate up to 87, 50, and 23 per cent, respectively. Spore viability is lost after storage for ten months on filter paper. By the vacuum filtered method, nearly all of the discharged spores can be captured. For quantitative recovery of spores, a filter such as the polycarbonate film would be more useful because of the greater back wash ability compared with cellulose filters like Whatman. Use of the polycarbonate filter would also allow direct microscopic observation of the collected spores. By operating the vacuum filter continuously, spore collections over various periods of time can be made in addition to single discharge collections (Steadman and Cook, 1974).

In another method empty injection vials (35 × 15 mm) are poured with water agar (1.5 per cent agar) and sterilized at 15 lb in.$^{-2}$ pressure for 15 min. Sclerotia bearing a single apothecium are planted on each vial aseptically, slightly below the neck of the vial. Each vial is subsequently inverted on a glass slide facing the apothecium. Apothecia can thus be kept safe from air currents. The whole set is incubated at 19–21°C under fluorescent light emitting 150–200 lux light intensity. The vial is lifted gently after 15–30 min of incubation and consequently the ascospores are puffed off vigorously from the apothecia forming a foggy appearance on the slide visible to the naked eye. Further release of ascospores that occurs after 2–3 h can be recovered on a fresh slide. The microscopic examination reveals the fogginess on the glass slide due to the huge quantity of ascospores that adhere on the slide. In earlier methods microscopic examination of the substrate used for collection of ascospores are essential to ensure the discharge of ascospores whereas in the present method, foggy impression on the slide is enough evidence of ascospores shooting. Such slides can be directly processed for germination for inoculation or for other studies (Singh and Singh, 1985).

22.7 Single Ascospore Isolation from Apothecium

Suspend an apothecium within and from the top of a large bell jar so that the hymenial disc faces downward. An open Petri dish containing nutrient agar is then placed within and at the base of the bell-jar to catch the spores that float

down. The apparatus is left thus overnight. Later, the Petri dish is examined microscopically and young cultures from individual spores are transferred to tubes. This method is laborious and favourable to contaminations. In the improved method, the lower two thirds of an apothecium are immersed in 95 per cent alcohol. Immediately a cloud of ascospores is ejected. Taking advantage of this action, a single ascospore is isolated simply by placing three Petri dishes containing nutrient agar near a vial with enough 95 per cent alcohol to cover the lower two thirds of the apothecium when this is dropped into the vial. In order not to interfere with spore dispersal, vials are selected that are not much taller than the fruiting bodies and in order to keep the apothecia in an upright position to avoid getting alcohol on the hymenium, vials are selected that are only slightly larger in diameter than the apothecia. Immediately following immersion and the ejection of a cloud of ascospores, a Petri dish is momentarily opened in the cloud. This is quickly repeated with the second and third Petri dish. Usually the last Petri dish catches only a few scattered spores free from contamination (Tapke, 1946).

22.8 Preservation of Ascospores

Ascospores can be collected by two ways to preserve for long time.

22.8.1 Collection of Ascospores in Water

Ascospores are collected in distilled water by removing the cover of Petri dishes containing apothecia and quickly covering the dish with a funnel attached to a vacuum-operated water trap. Within a few minutes, the spores are removed from the water by using a Millipore membrane filter (type GS, 47 µm). After drying overnight in a laboratory at 25°C and 10 per cent relative humidity, the membrane filters with the spores are placed in standard 20–30 ml screw cap vials. Half of the vials are approximately half full of calcium chloride pellets that are covered with cotton to prevent direct contact between the spores and the $CaCl_2$. Equal numbers of vials with and without $CaCl_2$ are stored at room temperature (25 ± 2°C), refrigerator temperature (2 ± 2°C) and in a freezer at 19 ± 1°C. Immediately and after 1, 2, 4, 8, 12 and 24 months, sectors of membrane filters stored under various conditions are soaked briefly in water and the ascospores are removed by scraping the submerged membrane with a spatula. A 0.5 ml suspension of ascospores are spread over the surface of PDA in a Petri dish and incubated at 24°C. The percentage of spores that germinated is determined by counting 200 spores after 6 h of incubation for the zero and one month samples and after 16 h of incubation for older spores (Hunter et al., 1982b).

22.8.2 Collection of Dry Ascospores

Ascospores are collected in a dry state directly on a Millipore filter (type HA 0.45 µm) by inverting a modified Millipore funnel over an opened Petri dish containing apothecia and applying a vacuum. The membrane filter with spores is held in a plastic Petri dish at 22–24°C and ambient relative humidity until spores are collected on four to five filter discs (one to three days). Each Petri dish is placed in a desiccators containing $CaCl_2$ and refrigerated. Immediately and after 0.5, 1, 9, 12, 24 and 48 months, small sectors of the membrane filters are soaked in water for a few minutes and the spores are dislodged with a camel's hair brush. Sub samples of the spore suspension are held in water and also placed on PDA on microscope slides. The percent spore germination is determined after 6–18 h of incubation at 23°C. Ascospores survive for longer periods when stored over $CaCl_2$ in the refrigerator or the freezer. More than 90 per cent of the ascospores of *Sclerotinia sclerotiorum* collected from laboratory produced apothecia, dries on Millipore membrane filters and stored over calcium chloride desiccant in a closed vessel survive for 24 months when refrigerated or frozen. A higher percentage of spores also survive without a desiccant in a freezer. With a desiccant, survival of freshly collected spores at 25°C is adequate for ascospore inoculum to be shipped to workers who lack the facilities or experience to germinate sclerotia carpogenically (Hunter et al., 1982b).

22.9 Selective Medium

Potato dextrose agar (PDA) is a suitable medium for growth of *S. sclerotiorum*. However, when plates with PDA are exposed outdoors, *Mucor* spp., *Rhizopus* spp., *Aspergillus* spp. and other fungi develop on the medium, thus interfering with the growth and enumeration of colonies of *S. sclerotiorum* which originate from ascospores. Pentachloronitrobenzene (PCNB) 75 per cent a.i., which is toxic to *S. sclerotiorum* and some other fungi, incorporated into PDA at 12.5, 25, 50, and 100 mg/l before pouring the medium into plates. Also, 2.50 mg/l dihydrostreptomycin (DS) is added to the medium. PDA containing 25 mg/l PCNB and DS can be used as the selective medium which inhibits fungal growth other than *S. sclerotiorum*. This medium is also useful for studies on dispersal of ascospores (Ben-Yephet and Bitton, 1985).

22.10 Purification of Seeds from Sclerotia

In the case of sunflower, seeds are kept in water at 35–37°C for 7–8 min and stirred. Sclerotia of white rot pathogen separate from the seeds, settle on the bottom of the container, which can be removed. Seeds with water are passed through an upper

opening on to a drying mesh and dried for 2.5–3 h at 30°C, for 30 min at 35°C and for 1 h at 37°C (Tripolka, 1977).

22.11 Detection of *Sclerotinia* by ELISA

The ELISA technique used by Walcz et al. (1985) for detection of *Sclerotinia* in sunflower plants showed good specificity and sensitivity in detecting "*Sclerotinia* antigen" at a concentration as low as 10 ng ml^{-1}. The pathogen is incubated on PDA (pH 5.7) for one week at 25°C and the mycelia are scraped with a scalpel from the surface. A modified Lilly-Barnett liquid medium (pH 5.7) is also used and the mycelia are collected by filtration. Growth of the fungus is scraped from three Petri-dish cultures with 15 ml phosphate buffer saline (PBS) (pH 7.2) containing 0.5 per cent formalin. The suspension is sonicated at 0°C for 5 min. Rabbits are immunized with this suspension into the marginal ear vein. Doses of 0.2, 0.5, 1.0 and 2.0 ml are given at four days intervals. The 2.0 ml dose is then repeated twice. Four days later the ELISA titer of the serum is tested and the rabbit is bled when the titer reaches 1:32,000 or higher. In the case of lower titers, the 2.0 ml dose is repeated until satisfactory titer is reached. Sera are stored at −20°C without preservative. Anti-*Sclerotinia* conjugate is prepared from an antiserum which gives an absorbance (A) value of 0.30 at dilution of 1:32,000. The anti-*Sclerotinia* rabbit serum is purified for 1 g G according to the method of Clark and Adams (1977). This purified 1 g G is coupled with the enzyme, horse radish peroxidase as described by Avrameas (1969).

In ELISA procedure polystyrene micro-test plates are coated with 100 µl per well of anti *Sclerotinia* 1 g G diluted 1:5,000 in coating buffer (0.79 g Na_2CO_3, 500 ml distilled water, pH 9.6). After overnight incubation at 4°C, the wells are emptied and washed 3 × with PHS containing 0.05 per cent Tween-20 (PBS-T). Then 100 µl plant extract (0.1 g of the plant is ground in 1 ml extracting buffer, (PBS-T with 2 per cent polyvinyl-pyrrolidone), PVP is added to the wells. Each sample is replicated twice. After incubating for 1 h at 30°C, the wells are washed again and 100 µl of the conjugate diluted 1:10,000 in PBS-T with 0.5 per cent bovine serum albumin (Fluka) is added. Again the plates are incubated for 1 h and washed and then 150 µl of substrate solution (30 ml citric acid buffer, pH 5.6), 10 mg o-phenylandiamine-dihydrochloride, OPD and 5 µl H_20_2 are added to the wells. The reaction is stopped after 5 min by adding 50 µl 4N H_2SO_4. Absorbances (A) values are recorded by a ELISA Reader at 492 nm on every plate positive (*Sclerotium homogenate*) and negative (extract of healthy plant) samples are replicated six times. 0.1 g of sclerotium is homogenized in 1 ml of extracting buffer. After 1 h of sedimentation, the supernatant is collected and used for the assay. When a sample gives an A value 3 × higher than the mean of the negative ones, it is considered to be positive. In practice, A values of negative samples remain below 0.20.

The reactivity of rabbit antisera is tested by a modified ELISA in the following sequence: (1) wells coated with the antigen (*Sclerotium* extract); (2) serial dilutions

of antisera (from rabbit immunized with *Sclerotinia*); (3) conjugate (anti-rabbit-l g G labelled with horse-radish peroxidase); (4) substrate.

Seeds of sunflower, soybean, bean and oil rape are sown in pots and inoculated through wounds on the stems at six to eight leaf stage of development with agar discs 0.5 mm diameter originating from *S. sclerotiorum* cultures maintained on PDA for a week. The sites of inoculation are covered with sterile wet cotton and aluminum foil. The infected plants are kept under alternating conditions of temperature and light (14–25°C, dark-daylight, respectively), in a greenhouse and sprayed with water, 8–12 plants of each species are inoculated and uninoculated ones are used as control. Stems and leaf pieces with early symptoms of disease and without symptoms are collected. Single pieces are put into nylon bags and coded. In the laboratory the samples are divided aseptically into two parts, one for culturing on PDA and the other for ELISA test. ELISA differentiates well between different fungi causing simultaneous infection with similar symptoms. PCR based assays for detecting inoculum of *Sclerotinia* spp. have been described by Freeman et al. (2002).

22.12 Medium for Production of Oxalic Acid

Three media used for production of oxalic acid were evaluated for their efficiency by Pierson and Rhodes (1992). The liquid media evaluated were:

(a) Potato dextrose broth.
(b) PDB with addition of 56 mM sodium succinate (PDB + S).
(c) Maxwell and Lumsden medium (M&L). It is prepared using the inorganic salts and yeast extract medium described by Maxwell and Lumsden (1970) with 23.7 mM D-glucose and 56 mM sodium succinate. The pH of the medium is not adjusted before autoclaving.

Media (125/250 ml Erlenmeyer flask) are autoclaved for 15 min at 121°C. A single ascospore isolate of *S. trifoliorum* is collected from red clover (*Trifolium pratense* L.) or alfalfa plot. Subcultures of this isolate are grown on potato dextrose agar acidified to pH 4 with 1 ml of 85 per cent lactic acid per litre of medium (APDA). Plugs of agar plus mycelium or agar only 8 mm in diameter are cut from three days old cultures or from uninoculated APDA plates which serve as controls. Three mycelial or three control plugs are transferred to each flask of liquid medium. Cultures are incubated at 15°C on a rotary shaker at 120 rpm with a 12 light/dark cycle provided by cool-white fluorescent lights (3,000–4,000 lux). Cultures are sampled 3, 5, 7, 10, 12 and 14 days after inoculation by removing 2 ml of medium per flask with a sterile Pasteur pipet. Samples are stored at −5°C prior to oxalic acid determination. After 14 days of incubation, the final pH of the medium is measured.

Oxalic acid concentrations are measured calorimetrically, oxalate, zirconium and quercetin solutions are prepared as described by Allan et al. (1986). An oxalic acid standard solution (1 mg/ml) is prepared after drying oxalic acid at 120°C for 2 h. To prepare a standard curve 0.8 ml zirconium solution, 0.8 ml quercetin solution, 0.5 ml

concentrated HCI, 0.1 ml of uninoculated (control) culture medium and 0.1 ml, 20, 30, 40, or 50 µl oxalic acid standard solution are added to a series of 10 ml volumetric flasks. Flasks are filled with double distilled water and allowed to incubate at 21°C for a minimum of 10 min. Absorbance of the solution at 430 nm is measured using a Spectronic-20. Linear regression is used to obtain standard curves. New standard curves are established each day and whenever new reagents are made.

To determine concentrations of oxalic acid in inoculated culture media, 0.1 ml of inoculated medium is substituted for control medium and oxalic acid. Inoculated medium is diluted with control medium if necessary. Oxalic acid determinations are made on a minimum of three aliquots per flask per sampling date. After 14 days of incubation, *S. trifoliorum* in PDB + S produces approximately 9–12 times more oxalic acid than in M&L or PDB, respectively.

22.13 Medium for Growth and Sporulation of *Sporidesmium sclerotivorum*

A method of cultivation and use of *S. sclerotivorum* as a biological control agent for plant disease has recently been patented (W. A. Ayers and P.D. Adams, U.S. Patent 4,246,258). *S. sclerotivorum* was initially cultivated *in vitro* on a medium derived from sclerotia of *S. minor (*Adams, 1979; Ayers and Adams, 1979a) and grew poorly on many common microbiological media (Ayers et al., 1981b). Substantially improved growth is obtained on media developed as a result of nutritional study (Barnett and Ayers, 1981). A mineral salts-glucose-glutamine medium supplemented with thiamine and biotin provides substantial yield of mycelium, especially when pH is controlled within the range of pH 5.0–5.5. Production of conidia, however, is frequently poor or erratic. Ayers and Adams (1983) determined additional nutritional and environmental factors affecting growth and sporulation of the beneficial mycoparasite *S. sclerotivorum*. A liquid medium composed of mineral salts, glucose, glutamine, thiamine, biotin and succinic acid supports good growth and sporulation of isolate CS-5 within five weeks at 25°C. Growth is substantially greater in a medium with KH_2PO_4, $CaCl_2$, minor elements and ferric-potassium salt of EDTA (Fe KEDTA) than in media with single omissions of these ingredients. Iron supplied as $FeCl_3$ or Fe KEDTA at 10–5 M or greater markedly stimulates production of conidia. Supplementation of the medium with casamine acids or soytone and yeast extract decreases the lag phase slightly but does not affect the maximum growth rate nor final mycelial and spore yields. Succinic acid (0.2 per cent) buffers the medium against a rapid drop in pH and therefore promotes growth. The growth rate is independent of glucose concentrations of 0.5–2 per cent, but the total mycelial yield is dependent upon the total amount of glucose furnished. Conidia develop primarily on mycelial mat surfaces, and maximum spore yields are favoured by cultivation in shallow depths of medium. The basal medium has the following composition per litre of distilled water: KH_2PO_4, 1 g; $MgSO_4.7H_2O$, 0.5 g; $CaCl_2.2H_2O$, 0.1 g; Succinic acid, 2 g; H_3BO_4, 2.86 µg; $MnCl_24H_2O$,1.41 µg;

$ZnSO_4.7H_2O$, 0.22 µg; $CuSO_4.5H_2O$, 0.08 µg $(NH_4)_6Mo_7O_{24}.H_2O$, 0.20 µg; ferric-potassium salt of ethylenediamine-tetra-acetic acid (Fe KEDTA), 9×10^{-5} M; thiamine hydrochloride, 100 µg and biotin, 10 µg. Medium SM^{-3} contains glucose, 10 g and L-glutamine, 1 g/l of basal medium. Medium SM^{-4} contains 5 g vitamin assay casamino acids/l in addition to the ingredients of SM^{-3}. Medium SM^{-6} contains 5 g soytone/l and 1 g yeast extract/l in addition to the ingredients of SM^{-3}. All media are adjusted to pH 5.3 with NaOH before autoclaving. Mycelial growth and production of conidia are determined in 250 ml Erlenmeyer flasks, capped with aluminum foil, containing various quantities of medium (25 ml) sterilized by autoclaving at 120°C for 20 min. Flasks of media are inoculated with 6 mm discs cut from colonies of *S. sclerotivorum* grown on SM^{-4} agar for four weeks. Cultures are incubated at 25 ± 2°C.

22.14 Use of Aerial Photography

The spectral, spatial and temporal characteristics of *Sclerotinia* blight can be detected by natural colour and false colour infra-red photography. Aerial photography depicts disease patterns which are difficult to observe from the ground. Disease areas, characterized by a unique spectral signature are best detected on false colour infra-red imagery. Moderate to severe disease infestations can be detected on false colour infra-red imagery photographed from 19,803 m above mean sea level. High altitude flights (19,803 m) are better for large area disease surveys, whereas low altitude flights (3,504 m) give better resolution for the detailed study of individual fields. Disease patterns that are difficult to observe from the ground and changes of the disease with time are easily detected by aerial photography. Imagery evaluation indicated that *Sclerotinia* blight was widespread in the Virginia peanut growing region during the 1974 growing season. Early detection of the disease via aerial surveys can permit diagnosis and control measures to minimize spread of the disease if chemical and/or other control measures are available. The imagery provides historical records of the disease. Study also provides a method of estimating the percentage of field affected by this disease in instances where the disease is considered to be moderate to severe (Powell et al., 1976).

22.15 Detection of Seed-Borne Infection

Seeds are soaked for 15 min and washed in flowing tap water for 30 min before being plated or planted. Seeds placed on 1 per cent water agar are observed for germination and emergence of microorganisms for one week. A seed is classified as germinated when the radical is 3 cm in length. Seeds from the same collections also are planted in sterilized soil in clay pots. Environmental conditions for development of white mould are optimized by holding these pots in humidity chambers at 18–20°C. The relative humidity in the chambers is 97–100 per cent for 16 h and

50–70 per cent for 8 h. After two weeks, percent germination and white mould infection is recorded. To confirm suspected white mould infection, tissue sections from diseased seedlings are plated on 1 per cent water agar and observed after one week for sclerotia of *Sclerotinia sclerotiorum* (Steadman, 1975). To detect mycelium in the infected seeds, seeds are surface sterilized for 2 min in 0.5 per cent sodium hypochlorite and then soaked in sterile water for 30 min. The seed coats are removed, cut into small pieces (2 mm^2) and examined under a microscope for the presence of dormant mycelium. The surface layers of the cotyledons are also cut into slices and examined similarly. After examination, all slides are kept in a moist chamber for 24 h and re-examined for mycelial growth (Tu, 1988). According to Koch and Menten (2000), the blotter test under incubation temperature of 15°C in the dark for 14 days is feasible quicker alternative for the detection of *S. sclerotiorum* in *P. vulgaris* seeds.

22.15.1 Semi-selective Media for Detection of Sclerotinia *on Bean and Soybean Seeds*

This work is aimed at evaluating the possibility of using bromophenol blue as an indicator for detecting the presence of *Sclerotinia sclerotiorum* in the seeds of dry-beans (*Phaseolus vulgaris*) and soybean (*Glycine max*), through incubation of the seeds on an agar medium and "blotter" substrates. The seeds are artificially inoculated with four *S. sclerotiorum* isolates, plated on the Neon agar medium and on modified Neon agar media, all incubated at 14°C and 20°C for seven days in the dark. Half of the seeds inoculated are surface disinfested prior to plating on the medium. The seeds showing change of colour in the medium, from blue to light yellow, as well as formation of typical mycelium and sclerotia in some cases are considered to be infected or contaminated by *S. sclerotiorum*. According to results obtained in this study, the Neon agar medium with incubation at 14°C or 20°C has proved to be a reliable and quick method for the detection of *S. sclerotiorum* mycelium in naturally infected seeds of bean and soybean (Peres et al., 2002).

22.15.2 Isolation and Determination of Incidence of Sclerotinia *in Peanut Seed*

Sclerotinia minor exists in peanut seed as dry mycelium and or sclerotia. Several fungi are commonly associated with peanut seed that may interfere with positive identification of *S. minor* from infected peanut seed. Soaking infected Okrun peanut seed in 1.05 per cent NaClO for 2 min reduces the number of contaminating fungi and increases the recovery of *S. minor*. Dry mycelia and sclerotia of *S. minor*

are submerged in 0, 0.26, 0.53, 1.05, 1.58, or 2.10 per cent aqueous solution of NaClO for 2 min, blotted dry and then plated on potato dextrose agar containing 100 µg/ml streptomycin sulfate (SPDA). A decrease in viability of dry mycelial fragments occurs with increasing concentration of NaClO, where 30 and 96 per cent inhibition of *S. minor* occurs at 0.26 and 2.10 per cent NaClO, respectively. There is no significant difference in viability of sclerotia that are submerged in the above concentration of NaClO. Okrun peanut seed naturally infected with *S. minor* is washed in 0.2 per cent liquid ivory soap, rinsed twice in deionized water and immersed in 0, 0.26, 0.53, 1.05, 1.58, or 2.10 per cent aqueous solution of NaClO for 1 min, air dried for 15 min, then plated onto SPDA. There is a reduction in the number of contaminating fungi isolated from seed exposed to concentrations greater than 0.53 per cent NaClO. Recovery of *S. minor* from naturally infested Okrun seed increases with NaClO concentrations up to 0.53 per cent. Okrun peanut seed infected with *S. minor* are sized as large, medium, or small by passing through 7.4 × 19.0 mm and 6.0 × 19.0 mm metal screens, respectively. Sized seed are submerged in 0.2 per cent liquid ivory soap, rinsed twice in deionized water and immersed in 1.05 per cent NaClO for 2 min air dried and plated on to SPDA. The infection of these seed ranges from 3.28 to 3.68 per cent and there is no significant difference between seed sizes in the per cent of seed infection with *S. minor* (Melouk et al., 1999).

22.16 Assessment of Losses Through Remote Sensing

By using a hand held Telatemp AG 42 IR thermometer a significant increase in leaf temperature can be observed in plants of oilseed rape infected with *Sclerotinia* stem rot. The increased canopy temperature due to disease induced water stress is of apparent importance in plant pathology. Studies of spectral reflectance of oilseed rape in the field plot experiment using a hand held Exotech 100AX radiometer (four wavelength bands: 500–600 nm, 600–700 nm, 700–800 nm and 800–1,100 nm) on two occasions after flowering (plant growth stage 5.1–5.3) has demonstrated a good correlation between spectral reflectance data, disease infection and yield of crude oil. Significant increases in reflectance of red light (R, 600–700 nm) and decreases in the two infra-red bands (IR, 700–800 nm and 800–1,100 nm) has been shown for the plots with moderate to severe infection by *Sclerotinia* stem rot and/or low level of N-fertilization in comparison to plots with slight infection and/or higher rate of N-fertilization. However, best discrimination is achieved in comparison of ratios between spectral reflectance of various wavelengths such as IR/R and (IR - R)/(IR + R) as well as the green/red ratio. The remote sensing method seems very promising as complements to conventional methods of early disease detection and assessment, as well as studies of plant growth and prognosis of yield (Nilsson, 1985).

22.17 RAPD-Based Molecular Diagnosis of Mixed Infections

Oilseed rape (*B. napus*) is attacked by parasitic fungi which often occur in mixed infections. Monitoring of these phytopathogens by morphological criteria is restricted due to their appearance especially in the later stages of disease development. Schleier et al. (1997) have developed molecular markers for a clear cut differentiation of a variety of rapeseed pathogenic fungi based on randomly amplified polymorphic DNA (RAPD). Twenty polymorphic fragments have been selected in southern hybridization experiments to test their taxon-specificity. Four amplification products give unspecific cross-hybridization patterns, one fragment corresponds to a genetic element common to three species within the genus *Alternaria* and 15 RAPD markers are highly specific for distinct fungal species. This report demonstrates the value of RAPD-PCR technique to amplify taxon-specific DNA fragments that can be used as hybridization probes for the diagnosis of a variety of rapeseed pathogens including *S. sclerotiorum*.

22.18 Cultivation of *Coniothyrium minitans*

The effects of growth media, temperature, pH and light on the development of four isolates of *Coniothyrium minitans* (CONIO and CH8, Colony type 3), G4 (colony type 4) and G9 (colony type 5) have been examined by McQuilken et al. (1997). Conidial germination, pycnidial production and hyphal extension rate were initially studied on seven different agar-based growth media at 18–20°C. Potato dextrose agar (PDA) and malt extract agar (MEA) consistently gives the greatest conidial germination, pycnidial production and hyphal extension rate for all four isolates. Growth and development on molasses yeast agar is equivalent to that on PDA and MEA except that hyphal extension rate is slower. Subsequently, the effects of temperature, pH and light on the development of *C. minitans* have been investigated on PDA only. The temperature range of conidial germination and pycnidial production of the four isolates is between 10–25°C with the optimum at approximately 20°C. Hyphal extension occurs over a greater temperature range, between 4°C and 25°C, with a maximum extension rate of approximately 3–5 mm d (−1) for all isolates occurring between 20–25°C. Conidial germination, pycnidial production and hyphal extension occurs over a pH range between 3–8 with optimum values for all growth assessments occurring between pH 4.5 and 5.6. Increasing light period from continuous dark, to 12 h light/12 h dark or continuous light has no effect on conidial germination or extension growth, but significantly increases pycnidial production. Isolates G4 and G9, previously characterized by sparse production of pycnidia in comparison with CONIO and CH8, consistently exhibit a reduced production of pycnidia on all media, at all temperatures and pH ranges and all light regimes tested. This demonstrates the stability of this character among these isolates of *C. minitans*.

According to McQuilken and Whipps (1995), *C. minitans* can be grown on solid substrates (barley, barley-rye-sunflower, bran-vermiculite, bran-sand, maize

meal-perlite, millets, oats, peat bran, rice and wheat) where it produces large numbers of germinable pycnidiospores ($1.9 - 9.3 \times 10^9$/g air dry inoculum). All solid substrate inocula survive better in the laboratory at 5°C and 15°C than at 30°C for at least 64 weeks. Soil incorporation of each inoculum almost completely inhibits carpogenic germination of *S. sclerotiorum*. Single pre-planting soil incorporation of five inocula (barley-rye-sunflower, maize-meal-perlite, peat bran, rice and wheat) controls *Sclerotinia* disease in a sequence of lettuce crops. At harvest, *C. minitans* reduces sclerotial population on the soil surface and >74 per cent of sclerotia recovered from plots treated with *C. minitans* are infected by the antagonist.

22.19 Immunoassay for Early Detection of *Sclerotinia sclerotiorum*

A serological test has been developed that allows the early detection of infection of young rapeseed petals by *S. sclerotiorum*. Two steps are required to obtain an antiserum sufficiently specific for *S. sclerotiorum*. Soluble mycelial extracts of *S. sclerotiorum* are used to produce the first generation polyclonal antiserum. This is not specific for *S. sclerotiorum* in DAS-ELISA and allows the screening of cross-reacting species such as *Botrytis cinerea* a pathogen commonly present on rapeseed petals. Using a polyclonal *anti B. cinerea* serum enables the absorption by serial cycles of *S. sclerotiorum* antigens common to *B. cinerea*. Residual antigens are then used as immunogens for the production of two second generation antisera (S_1 and S_2) which are then tested by DAS-ELISA. Cross-reactions with *B. cinerea* decrease with purification cycles of the immunogen whereas cross-reactions with some unrelated fungi slightly increase. *S. sclerotiorum* and *B. cinerea* are distinguishable using antiserum Sz (Jamaux and Spire, 1994). However, Lefol and Morrall (1996) developed immunofluorescent staining of *Sclerotinia* ascospores on canola petals. The quantitative relationship among mean numbers of ascospores/petal, the percentage of petals carrying ascospores as judged by immunofluorescent staining and the percentage of petals carrying viable ascospores as judged by a planting technique is studied by regression analysis. The multiple infection transformation successfully linearizes the relationship between per cent petals carrying ascospores and the mean numbers of ascospores per petal.

22.20 A Rapid Viability Test for Sclerotia

Four different fluorescent dyes viz., Acridine orange (AO), Fluorescin Diacetate (FDA), Calcofluor White MZR (CW) and Europium (E) Thenoyltrifluoroacetonate, 3-hydrate [Eu $(TT\ A)_3$] have been tested to distinguish rapidly between dead and living sclerotia from *S. trifoliorum*. FDA, 0.01 per cent diluted in phosphate buffer has the shortest staining period and is easy to handle. It is especially suitable to test large numbers of sclerotia for viability (Dittmer and Weltzien, 1990).

22.21 Artificial Incubation Method of Sclerotia

The quantity and quality of sclerotia produced by *S. sclerotiorum* in dark incubation on a medium of wheat bran + glucose + agar at 25°C for two days and then in incubation under light at 25°C are superior to that produced on other media and incubation methods. Potato and sweet potato used as substrates produces the highest quantity and quality of sclerotia, followed by carrot (Zhang-Yong Jie et al., 2004).

22.22 A Polymerase Chain Reaction (PCR) Assay for the Detection of Inoculum of *Sclerotinia sclerotiorum*

The development of a polymerase chain reaction (PCR) assay for the detection of inoculum of the plant pathogenic fungus *Sclerotinia sclerotiorum* is described. The PCR primers are designed using nuclear ribosomal DNA internal transcribed spacer sequences. Specific detection of DNA from *S. sclerotiorum* is possible even in the presence of a 40 fold excess of DNA from the closely related fungus *Botrytis cinerea*. PCR products are obtained from suspensions of untreated *S. sclerotiorum* ascospores alone, but DNA purification is required for detection in the presence of large numbers of *B. cinerea* conidiospores. Specific detection of inoculum of *S. sclerotiorum* is possible in field based air samples, using a Burkard spore trap and from inoculated oilseed rape petals. The assay has potential for incorporation into a risk management system for *S. sclerotiorum* in oilseed rape crops (Freeman et al., 2002).

22.23 Honeybee-Dispersed Biocontrol Agent to Manage Sunflower Head Rot

Efficacy of *Trichoderma* spp. to reduce sunflower head rot caused by *Sclerotinia sclerotiorum* has been evaluated in the field. A mixture of six isolates, including *Trichoderma koningii*, *T. aureoviride* and *T. longibrachiatum* has been tested in five field trials at Balcarce, Argentina. *Trichoderma* formulation (TF) included *Trichoderma* conidia and viable hyphal fragments, industrial talc and milled corn kernels. Honeybees (*Apis mellifera*) are used to disperse TF for six weeks from the onset of flowering. Two days after the first TF delivery, sunflower heads are inoculated with *S. sclerotiorum* ascospores. When 100 g TF is taken by honeybees in a 10-h per day period, head rot incidence is significantly reduced. This approach is successful in reducing disease incidence until physiological maturity of the crop, in environments highly conducive to head-rot development (Escande et al., 2002).

22.24 Assay of Bacterial Antagonistic Activity

Antifungal activity of bacterial strains is tested on solid as well as liquid medium (potato dextrose agar and broth). In plate assay, 50 µl of overnight grown cultures of bacterial strains in nutrient broth containing approximately 10^6 cells are inoculated in the centrally made well. Small plugs (5 mm diameter) of cultures of *S. sclerotiorum* inoculum from their leading edge are placed at four corners equidistant from the center. Plates are incubated at 30°C and zone of inhibition is measured. In liquid medium, reduction in fungal biomass of *S. sclerotiorum* is studied. *Pseudomonas maltophila* inoculum (10^4, 10^6 and 10^8 cells) is added to 72 h grown culture of the fungus in 25 ml potato dextrose broth. Reduction in mycelial biomass is also studied by addition of cell free culture filtrate (obtained by passing through a 0.45 µm membrane filter) of *P. maltophila* to 48 h grown cultures of *S. sclerotiorum*. The dry weight of mycelium is measures after filtering the contents through Whatman No. 1 filter paper and drying in an oven at 80°C (Kohli et al., 2006).

22.25 Use of Digital Imagery to Evaluate Disease Incidence and Yield Loss of Soybean

Remotely sensed spectral data have been used to assess the incidence of *Sclerotinia* stem rot of soybean caused by *S. sclerotiorum* and to determine its effect on soybean yields in a field in Waunakee, Wisconsin, USA. Multispectral data are obtained with an ATLAS sensor (Airborne Terrestrial Applications Sensor), yields are mapped with a combine-mounted yield monitor and field disease assessments are made both visually and by means of spectral reflectance observations obtained with a hand-held radiometer. Limitations in data obtained during the ground truth survey prevent use of multispectral data for disease assessment. However, the results suggested that disease incidence and crop yield can be estimated from spectral reflectance data, that plant disease can explain a high percentage of yield variability in a soybean field and that diseased areas can be mapped using precision agricultural techniques. It is suggested that this information will enable growers to use variable rate technologies to control *Sclerotinia* stem rot (Dudka et al., 1999).

22.26 Obtaining Pure *Sclerotinia sclerotiorum* Isolates from Contaminated Sclerotia

Pure cultures of different fungal species are necessary for many DNA investigations as well as polymorphism analysis of *S. sclerotiorum*. To obtain such pure cultures, plants susceptible to this pathogen and originating from interspecific crosses are used. Stem fragments of plants are cloned and thereafter inoculated with the pathogen.

Purification method is based on the process of reisolation of *S. sclerotiorum* mycelium from the upper part of stems previously infected with mixtures of unidentified fungi and bacteria obtained from preliminary isolation from sclerotia. With this method, contaminant free isolates are obtained and the presence of *S. sclerotiorum* is confirmed by microscopic observation of sclerotia and molecular marker RAPD method (Starzycka et al., 2001).

22.27 A PCR Assay for Detection of Carbendazim Resistance in *Sclerotinia sclerotiorum*

In recent years, resistance to carbendazim (MBC) in fungal pathogens has been attributed to single amino acid changes in the beta-tubulin subunit. The majority of these changes in field MBC-resistant isolates are located in amino acids 198 or/and 200. Part of the beta-tubulin gene from two *S. sclerotiorum* isolates with MBC resistant and sensitive phenotypes has been amplified using B1/B3 primers, cloned and sequenced. A point mutation at amino acid 198, causing a change from glutamic acid (GAG) to alanine (GCG), conferred MBC resistance in field. On the basis of this MBCHR mutation, two rapid detection methods were designed. The first relied on the creation of a ThaI restriction site (CGCG) at codons 197 and 198 (GACGAG - > GACGCG) in MBCHR isolate, in which ThaI cleaved the 874 bp amplification product of B1/B3 into 193 and 681 bp fragments, while products from MBCS isolate remained undigested. Two allele-specific oligonucleotides (ASO) with the codon 198 mutation at its terminal 3 base are synthesized and used in "nested" PCR or directly amplified from genomic DNA. The resistant and sensitive isolates are successfully detected by PCR amplification and ThaI restriction. These results are the same as those of mycelial growth tests (Li-Hong Xia et al., 2002).

22.28 Development of a Web-Based Forecasting Scheme

A number of monoclonal antibodies with potential for incorporation in rapid immunodiagnostic assay formats have been produced which are highly specific and sensitive to *Sclerotinia sclerotiorum*. As yet, the assay formats tested have not had the qualities of ease of use and accuracy required for a laboratory service or in-field methodology to be developed. The report identifies strategies for overcoming or avoiding problems such as the inhibition of the test caused by the presence of oilseed rape petal extracts in test samples through further development of the current protocols. Spatial analysis of *Sclerotinia* infected crops showed that the disease can occur in gradients across a field indicating an external inoculum source, or in 'hot spots' within the crop indicating a source of infection within the field. Data clearly emphasize the requirement for sampling at several points in each field for reliable determination of disease risk using agar or immunodiagnostic petal tests.

A sampling strategy involving taking samples on a 100 m grid pattern was able to detect the spatial variability present within the fields monitored. This is equivalent to approximately 15 samples for a 10 ha field. Further work is needed to confirm minimal, but statistically reliable, sampling strategies. Accurate prediction of inoculum and disease within a field may allow spraying to be targeted only in areas at risk. Field experiments showed that fungicide treatments were very effective (95 per cent control) for *Sclerotinia* control but timing of applications was critical. In 1999 and 2000, the optimum for sprays to control *Sclerotinia* was between late April and early May. However, the timing of infection was related to weather conditions favouring petal sticking and disease development and this was the critical factor determining the optimum growth stage within the flowering period for treatment. A difference of only ten days between treatments had a large effect on the level of disease control and this clearly identifies the value of a rapid test to guide spray applications. A range of risk factors for *Sclerotinia* stem rot have been investigated and together with data gathered have been incorporated into a web-based decision guide for determination of *Sclerotinia* disease risk. These factors include previous history of disease, weather and secondary crop factors. The guide also has options for inputting results of petal testing for estimation of inoculum potential. The decision guide calculates a numeric value for each set of data entered and assigns a value for risk of *Sclerotinia*, which is displayed as low, medium or high. The decision guide is available and now requires validation on farms (Turner et al., 2002).

22.29 Transformation of *Coniothyrium minitans* with *Agrobacterium tumefaciens*

Coniothyrium minitans is a potential biological control agent of the plant pathogenic fungus *Sclerotinia sclerotiorum*. T-DNA insertional transformation of strain ZS-1 of *C. minitans* mediated by *Agrobacterium tumefaciens* has been obtained with optimization of spore maturity for transformation. After confirmation by PCR, transformants are subjected to Southern blot analysis and results show that more than 82.7 per cent of transformants have single T-DNA insertions and 12.1 per cent of transformants have two copies T-DNA insertions. The genomic DNA segments of transformants flanking the T-DNA can be amplified from both borders with TAIL-PCR. Four types of mutants are screened and identified from the T-DNA insertional library, which comprised sporulation deficient mutants, pathogenicity deficient mutants, pigment change mutants and antibiotic deficient mutant and some of the mutants are described. The number and frequency of each type of mutant from the library have been calculated and the frequency of each type is 3.27×10^{-3}, 1.0×10^{-4}, 1.4×10^{-4}, 2.5×10^{-4}, respectively. The successful creation of the T-DNA insertional transformation library may help us to unravel the interaction between a parasite and its host at a molecular level, to clarify the differentiation and development of this fungus and to analyze and clone functional genes from the biocontrol microorganism in tripartite associations (Li-MoXiao et al., 2005).

Chapter 23
Future Strategies and Priorities

23.1 Future Strategies and Priorities for *Sclerotinia* Disease Management

It is not easy to set priorities of research for any biological system owing to its dynamic nature, plasticity and struggle for self survival. However, there is very thin line between haves and have nots, but following points may strengthen our *Sclerotinia* disease management package.

1. Biotechnological methods viz., gene transfer, *Agrobacterium tumefaciens* mediated transformation, protoplast culture, somatic hybridization and microplast techniques should be exploited for developing transgenic plants of crops with superior resistance to *Sclerotinia*.
2. Utilizing the benefits of *Agrobacterium* mediated transformation of bi-nucleate ascospores should expedite future gene transfer, gene knock-out and insertional mutagenesis studies on this pathogen.
3. Use of molecular techniques is required to resolve the question of phylogeny of *Sclerotinia* spp. other related genera and species.
4. Several strategies including detoxification defense, activation and general inhibition have potential to engineer *Sclerotinia* resistance.
5. Hypo virulent isolates of *Sclerotinia* spp. should be evaluated for their role in reducing virulence in populations and their potential application in disease management.
6. A better understanding of qualitative and quantitative attributes of *Sclerotinia* disease epidemics of different crops in variable environmental niche is required.
7. To make biological control a success, detailed studies are required on the ecology, biology, biochemistry, genetic engineering and molecular biology to understand mechanisms of action of BCAs through optional strain selection, improved formulation and delivery system.
8. Biological control products should be very effective (comparable to chemicals), economical, easy to use, non-toxic, environmentally safe to be acceptable to regulatory agencies, growers and consumers.

G.S. Saharan, Naresh Mehta, *Sclerotinia Diseases of Crop Plants: Biology, Ecology and Disease Management*,

9. The integrated disease management strategy including cultural, chemical, biological and host resistance should be refined, retested and revalidated under ever changing environmental conditions.
10. The knowledge of relationships between the growth stages of *Sclerotinia* and phenology of crops is effective in integrating management tactics that target the most vulnerable stages of the pathogen and the crop attributes that contribute to disease development in the field.
11. It may be promising to investigate potential disease avoidance mechanisms such as open and upright plant architecture as a source for selecting new cultivars in crops.
12. It is essential to establish inter and interdisciplinary team work between plant pathologists, microbiologists, molecular biologist, weed ecologists, agronomists, soil scientists, formulation experts and application technologists to overcome the constraints being faced for management of *Sclerotinia* diseases and to use this pathogen as mycoherbicide for obnoxious weed management.

References

Abawi, G. S. and Grogan, R. G. 1975. Source of primary inoculum and effects of temperature and moisture on infection of beans by *Whetzelinia sclerotiorum. Phytopathology* 65: 300–309.

Abawi, G. S. and Grogan, R. G. 1979. Epidemiology of diseases caused by *Sclerotinia* species. *Phytopathology* 69: 899–904.

Abawi, G. S., Polach, F. J. and Molin, W. T. 1975a. Infection of bean by ascospores of *Whetzelinia sclerotiorum. Phytopathology* 65: 673–678.

Abawi, G. S., Provvidenti, R., Grogan, R. G. and Hunter, J. E. 1975b. Predisposition of beans to infection by ascospores of *Whetzelinia sclerotiorum* prior to blossoming. *Proc. Am. Phytopathol. Soc*. 2: 61.

Abawi, G. S., Provvidenti, R., Crosier, D. C. and Hunter, J. E. 1978. Inheritance of resistance to white mold disease in *Phaseolus coccineus. J. Hered*. 69: 200–202.

Abawi, G. S., Robinson, R. W., Cobb, A. C. and Shail, J. W. 1980. Reaction of lettuce germ-plasm to artificial inoculation with *Sclerotinia minor* under greenhouse conditions. *Plant Dis*. 64: 668–671.

Abd-Elrazik, A. A. and Lorbeer, J. W. 1980. Rapid separation of *Sclerotinia minor s*clerotia from artificially and naturally infected organic soil. *Phytopathology* 70: 892–894.

Abu Dieyeh, M. H., Bernier, J. and Watson, A. K. 2005. *Sclerotinia minor* advances fruiting and reduces germination in dandelion (*Taraxacum officinale*). *Biocontrol Sci. Technol*. 15: 815–825.

Acimovic, M. 1979. Laboratory examination of some fungicides used in the control of *Sclerotinia libertiana* Fuckel. The parasite of sunflower. *Rev. Plant Pathol*. 38: 2332 (Abstr.).

Acuna, P. R., Latorre, G. B. and Cancino, E. L. 1976. Translocation of systemic fungicides in sunflower (*Helianthus annuus* L) and its action on *Sclerotinia sclerotiorum* (Lib.) de Bary. *Rev. Plant Pathol*. 33: 1137 (Abstr.).

Adams, P. B. 1975. Factors affecting survival of *Sclerotinia sclerotiorum* in soil. *Plant Dis. Reptr*. 59: 599–603.

Adams, P. B. 1979. A rapid method for quantitative isolation of sclerotia of *Sclerotinia minor* and *S. cepivorum* from soil. *Plant Dis. Reptr*. 63: 349–351.

Adams, P. B. 1986. Production of sclerotia of *Sclerotinia minor* on lettuce in the field and their distribution in soil disking. *Plant Dis*. 70: 1043.

Adams, P. B. 1987a. Comparison of isolates of *Sporidesmium sclerotivorum in vitro* and in soil for potential as active agents in microbial pesticides. *Phytopathology* 77: 575.

Adams, P. B. 1987b. Effect of soil temperature, moisture and depth on survival and activity of *Sclerotinia minor, Sclerotium cepivorum* and *Sporidesmium sclerotivorum. Plant Dis*. 71: 170–174.

Adams, P. B. 1989. Comparison of antagonists of *Sclerotinia* species. *Phytopathology* 79: 1345–1347.

Adams, P. B. and Ayers, W. A. 1979. Ecology of *Sclerotinia* species. *Phytopathology* 69: 896–899.

Adams, P. B. and Ayers, W. A. 1980. Factors affecting parasitic activity of *Sporidesmium sclerotivorum* on sclerotia of *Sclerotinia minor* in soil. *Phytopathology* 70: 366–368.

Adams, P. B. and Ayers, W. A. 1981. *Sporidesmium sclerotivorum* distribution and functions in natural biological control of sclerotial fungi. *Phytopathology* 71: 90–93.

Adams, P. B. and Ayers, W. A. 1982. Biological control of *Sclerotinia* lettuce drops by *Sporidesmium sclerotivorum. Phytopathology* 72: 485–488.

Adams, P. B. and Ayers, W. A. 1983. Histological and physiological aspects of infection of sclerotia of two *Sclerotinia* species by two mycoparasites. *Phytopathology* 73: 1072–1076.

Adams, P. B. and Ayers, W. A. 1985. The world distribution of the mycoparasites *Sporidesmium sclerotivorum*, *Teratosperma oligocladium* and *Laterispora brevirama. Soil Biol. Biochem.* 17: 155.

Adams, P. B. and Fravel, D. R. 1990. Economical biological control of *Sclerotinia* lettuce drops in the field by *Sporidesmium sclerotivorum. Phytopathology* 80: 1120–1124.

Adams, P. B. and Fravel, D. R. 1993. Dynamics of *Sporidesmium*, a naturally occurring mycoparasite. In: *Pest Management: Biologically Based Technologies* (R. D. Lumsden and J. L. Vaughn, eds.). ACS, Washington, DC, pp. 189–195.

Adams, P. B. and Tate, C. J. 1975. Factors affecting lettuce drop caused by *Sclerotinia sclerotiorum. Plant Dis. Reptr*. 59: 140–143.

Adams, P. B. and Tate, C. J. 1976. Mycelial germination of sclerotia of *Sclerotinia sclerotiorum* on soil. *Plant Dis. Reptr*. 60: 515–518.

Adams, P. B. and Wong, A. L. 1991. The effect of chemical pesticides on the infection of sclerotia of *Sclerotinia minor* by the biocontrol agent *Sporidesmium sclerotivorum. Phytopathology* 81: 1340–1343.

Adams, P. B., Tate, C. J., Lumsden, R. D. and Meissers, J. P. 1973. Resistance of *Phaseolus* species to *Sclerotinia sclerotiorum. Reptr. Bean. Improv. Coop*. 16: 8–9.

Adams, P. B., Marois, J. J. and Ayers, W. A. 1984. Population dynamics of the mycoparasite *Sporidesmium sclerotivorum* and its host *Sclerotinia minor* in soil. *Soil Biol. Biochem.* 16: 627.

Adams, P. B., Ayers, W. A. and Marois, J. J. 1985. Energy efficiency of the mycoparasite *Sporidesmium sclerotivorum in vitro* and in soil. *Soil Biol. Biochem*. 17: 155.

Aertsens, F. and Michi, H. 2004. *Coniothyrium minitans*: a soil fungus against *Sclerotinia* in multiple crops. *Phytoma* 571: 33–35.

Afek, U. and Carmeli, S. 1995. Increasing celery resistance to pathogens during storage and reducing high-risk psoralens concentration by treatment with GA3. *J. Am. Soc. Hort. Sci*. 120: 562–565.

Afek, U., Aharoni, N. and Carmeli, S. 1996. The involvement of marmesin in celery resistance to pathogens during storage and the effect of temperature on its concentration. *Phytopathology* 85: 711–714.

Aggarwal, R. A. K., Kumar, A. and Thakur, H. L. 1997. Effect of *Sclerotinia* rot on oil quality in low erucic acid cultivars of rapeseed. *Cruciferae Newslett*. 19: 103–104.

Agrios, G. N. 2005. *Plant Pathology*. 5th ed. Elsevier Academic, New York, 922 p.

Aguero, M. E., Pereyra, V. R. and Escande, A. R. 2001. Effect of sunflower head rot [*Sclerotinia sclerotiorum* (Lib.) de Bary] on impurities in harvested product and oil content and acidity. *J. Agric. Univ. Puerto Rico* 85: 177–186.

Akai, J. 1981. Studies on the epidemiology and control of *Sclerotinia* disease of beans caused by *Sclerotinia sclerotiorum* (Lib.) de Bary. *Hokkaido Prefectural Agr. Exp. Stn. Reptr*. 36: 77–79.

Akem, C. N. and Melouk, H. A. 1985. Colonization of sclerotia of *Sclerotinia minor* by a potential biocontrol agent *Penicillium citrinum. Peanut Sci*. 14: 66–70.

Akem, C. N. and Melouk, H. A. 1990. Transmission of *Sclerotinia minor* in peanut from infected seed. *Plant Dis*. 74: 216–219.

Akem, C. N., Melouk, H. A. and Smith, O. D. 1985. Field evaluation of peanut genotypes for resistance to *Sclerotinia* blight. *Crop Prot*. 11: 345–348.

Alabouvette, C. and Louvet, J. 1973. *Sclerotinia sclerotiorum*. Bibliographie Sclerotionnee (Dec. 1971). *Inform. Tech. Centre Tech. Interprof. Oleag. Metrop*. 31: 7–26.

Aldrich, D. T. A. 1974. The legume - A reappraisal of its place in today's farming. *J. Br. Grassland Soc*. 24: 249.

Ale-Agha, N. 1974. A study on *Sclerotinia sclerotiorum* (Lib.) de Bary in Iran. *Ann. Phytopathol.* 6: 385.

Alighisi, P. and Favaron, F. 1995. Pectin degrading enzymes and plant parasite interactions. *Eur. J. Plant Pathol*. 101: 365–375.

Alister, C. and Trotus, E. 1989. Studies of the effects of some agro technical factors on prevention and control of *Sclerotinia sclerotiorum*. (Lib.) de Bary on sunflower. *Cercetori Agronomice in Moldova* 22: 63–66.

Allan, A. L., Fernandez, B. and Robio, B. S. 1986. Spectrophotometer determination of oxalate in aqueous solution. *Microchem. J*. 34: 51–55.

Aloj, B., Scalcione, M., Nanni, B. and Pugliano, G. 1994. Observations on some rots of fruits of Feyoa. 11. *Annali dalla Facolta di Geieuze Agrarie della Universita degli Gtudi di Napoli Partrei* 28: 36–47.

Anas, O. and Reeleder, R. D. 1987. Recovery of fungi and arthropods from sclerotia of *Sclerotinia sclerotiorum* in Quebec muck soils. *Phytopathology* 77: 327–331.

Anas, O. and Reeleder, R. D. 1988a. Feeding habits of larvae of *Bradysia coprophila* on fungi and plant tissue. *Phytoprot*. 69: 73–78.

Anas, O. and Reeleder, R. D. 1988b. Consumption of sclerotia of *Sclerotinia sclerotiorum* by larvae of *Bradysia coprophila:* Influence of soil factors and interactions between larvae and *Trichoderma viride. Soil Biol. Biochem*. 20: 619–624.

Anas, O., Alli, I. and Reeleder, R. D. 1989. Inhibition of germination of *Sclerotinia sclerotiorum* by salivary glands secretions of *Bradysia coprophila. Soil. Biol. Biochem*. 21: 47–52.

Anderson, F. N., Steadman, J. R., Coyne, D. P. and Schwartz, H. F. 1974. Tolerance to white mould in *Phaseolous vulgaris* dry edible bean types. *Plant Dis. Reptr*. 58: 782–784.

Anderson, J. B. and Kohn, L. M. 1995. Clonality in soil borne plant pathogenic fungi. *Annu. Rev. Phytopathol*. 33: 369–391.Anderson, T. R. 1985. Seed molds of soybean in Ontario and the influence of production area on the incidence of *Diaporthe phaseolarum* var. *caulivara* and *Phomopsis* sp. *Can. J. Plant Pathol*. 7: 74–78.

Andrews, J. H. 1992. Biological control in the phyllosphere. *Ann. Rev. Phytopathol*. 30: 603–635.

Annis, S. L. and Goodwin, P. H. 1997. Recent advances in molecular genetics of plant cell wall degrading enzymes in plant pathogenic fungi. *Eur. J. Plant Pathol*. 103: 1–14.

Anonymous. 1931. Carrot. *Can Plant Dis. Surv*. 37.

Anonymous. 1952. First latin American congress of phytopathology. *Rev. Appl. Mycol*. 31: 372 (Abstr.).

Anonymous. 1968a. Distribution Maps of Plant Diseases *Sclerotinia narcissicola* Map No. 315, June (Edition 2), CAB International, Nosworthy Way, Wallingford, Oxfordshire, OX10 8DE.

Anonymous. 1968b. Distribution Maps of Plant Diseases *Sclerotinia borealis* Map No. 446, October (Edition 1), CAB International, Nosworthy Way, Wallingford, Oxfordshire, OX10 8DE.

Anonymous. 1970. Carrot. *Can Plant Dis. Surv*. 50: 20.

Anonymous. 1976a. Distribution Maps of Plant Diseases *Sclerotinia fructigena* Map No. 22, October (Edition 4), CAB International, Nosworthy Way, Wallingford, Oxfordshire, OX10 8DE.

Anonymous. 1976b. Distribution Maps of Plant Diseases *Sclerotinia laxa* Map No. 44, April (Edition 4), CAB International, Nosworthy Way, Wallingford, Oxfordshire, OX10 8DE.

Anonymous. 1976c. Distribution Maps of Plant Diseases *Sclerotinia fructicola* Map No. 50, April (Edition 4), CAB International, Nosworthy Way, Wallingford, Oxfordshire, OX10 8DE.

Anonymous. 1977. Distribution Maps of Plant Diseases *Sclerotinia squamosa* Map No. 164, April (Edition 4), CAB International, Nosworthy Way, Wallingford, Oxfordshire, OX10 8DE.

Anonymous. 1979. Control of fruit rot of cucurbitaceae (*Botrytis cinerea – Sclerotinia sclerotiorum*) *Ravue Horticole*. 200: 35 [Fv.].

Anonymous. 1982. Distribution Maps of Plant Diseases *Sclerotinia trifoliorum* Map No. 274, April (Edition 3), CAB International, Nosworthy Way, Wallingford, Oxfordshire, OX10 8DE.

Anonymous. 1998. Distribution Maps of Plant Diseases *Sclerotinia fuckeliana* Map No. 22, October (Edition 4), CAB International, Nosworthy Way, Wallingford, Oxfordshire, OX10 8DE.

Anonymous. 2003. Distribution Maps of Plant Diseases *Sclerotinia minor* Map No. 889, April (Edition 1), CAB International, Nosworthy Way, Wallingford, Oxfordshire, OX10 8DE.

Anonymous. 2005a. Distribution Maps of Plant Diseases *Sclerotinia sclerotiorum* Map No. 971, October (Edition 1), CAB International, Nosworthy Way, Wallingford, Oxfordshire, OX10 8DE.

Anonymous. 2005b. *Sclerotinia* Initiative Brochure. United States Department of Agriculture, Fargo, ND. www.whitemoldresearch.com.

Antokolskaya, M. P. 1927. Notes on *Sclerotinia libertiana* on the sunflower. *Rev. Appl. Mycol*. 6: 330 (Abstr.).

Arahana, V. S., Graef, G. L., Specht, J. E., Steadman, J. R. and Eskridge, K. M. 2001. Identification of QTLs for resistance to *Sclerotinia sclerotiorum* in soybean. *Crop Sci*. 41: 180–188.

Arseniuk, E. 1989. Effect of induced autotetrapoloidy on response to *Sclerotinia* clover rot in *Trifolium pratense* L. *Plant Breed*. 103: 310–318.

Arseniuk, E. and Macewiez, J. 1994a. Scanning electron microscopy of apothecia of *Sclerotinia trifoliorum* Erikss. and related species. *J. Phytopathol*. 141: 267–274.

Arseniuk, E. and Macewiez, J. 1994b. Scanning electron microscopy of sclerotia of *Sclerotinia trifoliorum* Erikss. and related species. *J. Phytopathol*. 141: 275–284.

Ashton, F. M. and Crafts, A. S. 1981. *Mode of Action of Herbicides*. Wiley, New York, p. 525.

Asirifi, K. N., Morgan, W. E. and Parbecy, D. G. 1994. Suppression of *Sclerotinia* soft rot of lettuce with organic soil amendments. *Aust. J. Exptl. Agri*. 24: 131–136.

Atallah, Z. K. and Johnson, D. A. 2004. Development of *Sclerotinia* stem rot in potato fields in South-Central Washington. *Plant Dis*. 88: 419–423.

Atallah, Z. K., Larget, B., Chen, X. and Johnson, D. A. 2004. High genetic diversity, phenotypic uniformity and evidence of out crossing in *Sclerotinia sclerotiorum* in the Columbia basin of Washington State. *Phytopathology* 94: 737–742.

Auclair, J., Boland, G. J., Kohn, L. M. and Rajcan, I. 2004a. Genetic interactions between *Glycine max* and *Sclerotinia sclerotiorum* using a straw inoculation method. *Plant Dis*. 88: 891–895.

Auclair, J., Boland, G. J., Cober, E., Graef, G. L., Steadman, J. R., Zilka, J. and Rajcan, I. 2004b. Development of a new field inoculation technique to assess partial resistance in soybean to *Sclerotinia sclerotiorum. Can. J. Plant Sci*. 84: 57–64.

Auger, S. J. and Nome, H. F. 1971. Action of the systemic fungicides Benlate 50% (1 buty1 carbamoyl-2-benzimidazole carbauric acid, methyl-ester) on *Sclerotinia sclerotiorum* casual agent of sunflower wilt. *Rev. Plant Pathol*. 50: 2096 (Abstr.).

Avila, de Moreno, C. and Gutierrez, de Gerardino, A. 1992. Biological control of *Sclerotinia sclerotiorum* (Lib.) de Bary on lettuce (*Lactuca sativa* L.). *Fitopatologia Colombiana* 16: 172–179.

Avrameas, S. 1969. Coupling of enzymes to proteins with glutaraldehyde, use of conjugates for the detection of antigens and antibodies. *Immunochemistry* 5: 43.

Ayers, W. A. and Adams, P. B. 1979a. Mycoparasitism of sclerotia of *Sclerotinia* and *Sclerotium* species by *Sporidesmium sclerotivorum. Can. J. Microbiol*. 25: 17–23.

Ayers, W. A. and Adams, P. B. 1979b. Factors affecting germination, mycoparasitism and survival of *Sporidesmium sclerotivorum. Can. J. Microbiol*. 25: 1021–1026.

Ayers, W. A. and Adams, P. B. 1981a. Mycoparasitism and its application to biological control of plant diseases. In: *Biological Control in Crop Production* (G. C. Papavizas, ed.). BARC Symposium Vol. 5, Allanheld Osmum, Totowa, NJ, pp. 91–103.

Ayers, W. A. and Adams, P. B. 1981b. Mycoparasitism of sclerotia fungi by *Teratosperma oligocladium. Can J. Microbiol*. 27: 886–892.

Ayers, W. A. and Adams, P. B. 1983. Improved media for growth and sporulation of *Sporidesmium sclerotivorum. Can. J. Microbiol*. 29: 325–330.

Ayers, W. A. and Adams, P. B. 1985. Interaction of *Laterispora brevirama* and the mycoparasites *Sporidesmium sclerotivorum* and *Teratosperma oligocladium. Can. J. Microbiol*. 31: 786.

Ayers, W. A., Barnett, B. A. and Adams, P. B. 1981. Germination of micro-conidia and growth of *Sporidesmium sclerotivorum in vitro. Can. J. Microbiol*. 27: 664–669.

Backhouse, D. and Willetts, H. J. 1984. A histochemical study of *Botrytis cinerea* and *Botrytis fabae. Can. J. Microbiol*. 30: 171–178.

Bacon, J. S. D., Grogan, A. H. and Jones, D. 1972. Degradation of sclerotia, a β-(1–3) glucan by enzymes from fungi parasitic on sclerotia. *Biochem. J*. 129: 27–28.

Baibakova, O. V. 1989. Identification of forms of sunflower resistant to *Sclerotinia sclerotiorum*. In: *Zashchita rastenii v intensivnoi tekhnologii vozdelyvaniya sel skokho zyaistvennykh kul'tur*. Voronezh, pp. 145–149.

Bag, T. K. 2003a. *Sclerotinia sclerotiorum* on butterfly flower-a new record. *Indian Phytopath*. 56: 121.

Bag, T. K. 2003b. Occurrence of *Sclerotinia* blight of mungbean and urdbean under rainfed conditions of Arunachal Pradesh. *J. Mycopathol. Res*. 41: 43–46.

Bag, T. K. 2006. A new report on *Sclerotinia* white rot of two jewel orchids from Darjeeling India. *J. Mycol. Pl. Pathol*. 36: 304.

Bains, P. S., Bennypaul, H. S., Blade, S. F. and Weeks, C. 2000. First report of hemp canker caused by *Sclerotinia sclerotiorum* in Alberta. *Can. Plant Dis*. 84: 372–372.

Baker, K. F. and Davis, L. H. 1951. An unusual occurrence of sclerotia of *Sclerotinia* species with seed of *Centaurea cygnus. Plant Dis. Reptr*. 35: 39–41.

Baldini, M., Turi, M., Vischi, M., Vannozzi, G. P. and Olivieri, A. M. 2001. Evaluation of genetic variability for *Sclerotinia sclerotiorum* (Lib.) de Bary resistance in sunflower and utilization of associated molecular markers. *SUNBIO* 2001. In*: Proceedings of Vth European Conference on Sunflower Biotechnology, San Giuliana Terme, Pisa*, Italy, 4–8 November, 2001. *Helia* 25: 177–189.

Baraer, J. 1979. Protection against *Botrytis cinerea* and *Sclerotinia sclerotiorum* of vegetable crops for canning. *Ravue Horticole* 198: 53–54 (Fr.)

Barkai-Golan, R. 1974. Production of cellulose and poly-galacturonase by *Sclerotinia minor. Mycopathol. Mycol. Appl*. 54: 297–302.

Barnett, E. A. and Ayers, W. A. 1981. Nutritional and environmental factors affecting growth and sporulation of *Sporidesmium sclerotivorum. Can. J. Microbiol*. 27: 685–691.

Basse, C. and Steinberg, G. 2004. *Ustilago maydis*, a model system for analysis of the molecular basis of fungal pathogenicity. *Mol. Plant Pathol*. 4: 83–92.

Baswana, K. S., Rastogi, K. B. and Sharma, P. P. 1991. Inheritance of stalk rot resistance in cauliflower (*Brassica oleracea* var. *botrytis* L). *Euphytica* 57: 93–96.

Bateman, D. F. 1964. An induced mechanism of tissue resistance to polygalacturonase in *Rhizoctonia* infected hypocotyls of bean. *Phytopathology* 54: 438–445.

Bateman, D. F. and Beer, S. V. 1965. Simultaneous production and synergistic action of oxalic acid and polygalacturonase during pathogenesis by *Sclerotinia rolfsii. Phytopathology* 55: 204–211.

Bauer, W. D., Bateman, D. F. and Whalen, C. H. 1977. Purification of an endo-β-l, 4 galactanase produced by *Sclerotinia sclerotiorum:* effects on isolated plant cell-walls and potato tissue. *Phytopathology* 67: 862–868.

Beach, W. S. 1921. The lettuce “drop” due to *Sclerotinia minor. PA Agric. Exp. Stn. Bull*. 165: 27.

Becelaere, G. van and Miller, J. F. 2004. Methods of inoculation of sunflower heads with *Sclerotinia sclerotiorum. Helia* 27: 137–142.

Bedi, K. S. 1958. Effect of ultra-violet radiation of sclerotia of *Sclerotinia sclerotiorum* de Bary on speed of their germination and apothecial development. *Indian Phytopath*. 11: 37–39.

Bedi, K. S. 1962. Light, air and moisture in relation to the formation of apothecia of *Sclerotinia sclerotiorum* (Lib.) de Bary. *Proc. Indian Acad. Sci*. 55: 213–223.

Bedi, K. S. 1963. The age and the size of sclerotia of *Sclerotinia sclerotiorum* (Lib.) de Bary in relation to the formation of apothecia. *Indian Bot. Soc*. 42: 204–207.

Behboudi, K., Sharifi-Tehrani, A., Hedjaroude, G. A., Zad, J., Mohammadi, M. and Rahimian, H. 2005. Effects of fluorescent *Pseudomonas* on *Sclerotinia sclerotiorum*, the causal agent of sunflower root rot. *Iran. J. Agril. Sci*. 36: 791–803.

Bell, A. A. and Wheeler, M. H. 1986. Biosynthesis and functions of fungal melanins. *Annu. Rev. Phytopathol*. 24: 411–451.

Bell, A. A., Liu, L., Reidy, B., Davis, R. M. and Subbarao, K. V. 1998. Mechanism of subsurface drip irrigation-mediated suppression of lettuce drop caused by *Sclerotinia minor. Phytopathology* 38: 252–259.

Bennett, O. L. and Elliot, E. S. 1972. Plant disease incidence on five forage species as affected by North and South facing slopes. *Plant Dis. Reptr*. 56: 371–375.

Benoist, M. 1979. Control of attacks of *Botrytis* and *Sclerotinia* on haricot bean and *Botrytis* on pea. *Ravue Horticole* 198: 55 (Fr.).

Ben-Yephet, Y. 1988. Control of sclerotia and apothecia of *Sclerotinia sclerotiorum* by metham-sodium, methyl bromide and soil solarization. *Crop Prot*. 7: 25–27.

Ben-Yephet, Y. and Bitton, S. 1985. Use of selective medium to study the dispersal of ascospores of *Sclerotinia sclerotiorum. Phytoparasitica* 13: 33–40.

Berkenkamp, B. and Friesen, H. A. 1973. Effects of barban on stem rot of rape. *Can. J. Plant Sci*. 53: 917.

Bert, P. F., Dechamp-Guillaume, G., Serre, F., Jouan, I., Tourvieille de Labrouhe, D., Nicolas, P. and Vear, F. 2004. Comparative genetic analysis of quantitative traits in sunflower (*Helianthus annuus* L.): 3. Characterization of QTL involved in resistance to *Sclerotinia sclerotiorum* and *Phoma macdonaldi. Theor. Appl. Genet*. 109: 865–874.

Berthet, P. 1964 Essai Biotaxonomique sur les Discomycetes. *These Faculte des Sciences de l' Universite de Lyon.* 157 p. Berthet, Lyon.

Beute, M. K., Porter, D. M. and Hadley, B. A. 1975. *Sclerotinia* blight of peanut in North Carolina and Virginia and its chemical control. *Plant Dis. Reptr*. 59: 697–700.

Bisby, G. R. 1921. Stem rot of sunflower in Manitoba. *Sci. Agric*. 2: 58.

Bisby, G. R. 1924. The *Sclerotinia* disease of sunflower and other plants. *Sci. Agric*. 4: 381.

Bjorling, K. 1942. Undersokninger rorande kioverrotan. II Studier av utvecklingshistoria och variation hos *Sclerotinia trifoliorum. Meddland Statens Vaxtskyddsanst* 37: 1–154.

Bjorling, K. 1951. Uber die entwicklungsgeschiehte, variabilitat and pathogenitat von *Sclerotinia trifoliorum* Erikss. *Phytopathologische Zeitschrift* 18: 129–156.

Blad, B. and Steadman, J. R. 1975. Relationship of microclimate and white mold disease in dry bean crops as influenced by irrigation and canopy structure. *Agron*.: 10 (Abstr.).

Blad, B. and Steadman, J. R. and Weiss, A. 1978. Canopy structure and irrigation influence white mold disease and microclimate of dry edible beans. *Phytopathology* 68: 1431–1437.

Blakeman, J. P. 1985. Ecological succession of leaf surface microorganisms in relation to biological control. In: *Biological Control on the Phylloplane* (C. E. Windels and S. E. Lindow, eds.). American Phytopathological Society, St. Paul, MN.

Blanchette, B. L. and Auld, D. L. 1978. Screening field peas for resistance to white mould. *Crop. Sci*. 18: 977–979.

Blodgett, E. C. 1946. The *Sclerotinia* rot disease of beans in Idaho. *Plant Dis. Reptr*. 30: 137–144.

Bohar, G. and Kiss, L. 1999. First report of *Sclerotinia sclerotiorum* on common ragweed (*Ambrosia artemisiifolia*) in Europe. *Plant Dis*. 83: 302.

Boland, G. J. 1987. Hypovirulence, debilitation and double-stranded RNA in *Sclerotinia sclerotiorum. Phytopathology* 77: 1613.

Boland, G. J. 1988. Hypovirulence in *Sclerotinia sclerotiorum* associated with double stranded RNA. *Plant Pathol*. 10: 361.

Boland, G. J. 1992. Hypovirulence and double-stranded RNA in *Sclerotinia sclerotiorum. Can. J. Plant Pathol*. 14: 10–17.

Boland, G. J. 2004. Fungal viruses, hypo-virulence and biological control of *Sclerotinia* species. *Can. J. Plant Pathol*. 26: 6–18.

Boland, G. J. and Hall, R. 1987. Evaluating soybean cultivars for resistance to *Sclerotinia sclerotiorum* under field conditions. *Plant Dis*. 71: 934–936.

Boland, G. J. and Hall, R. 1988a. Numbers and distribution of apothecia of *Sclerotinia sclerotiorum* in relation to white mould of white bean (*Phaseolus vulgaris*). *Can. J. Bot*. 66: 247–252.

Boland, G. J. and Hall, R. 1988b. Relationships between the spatial pattern and number of apothecia of *Sclerotinia sclerotiorum* and stem rot of soybean. *Plant Pathol*. 37: 329–336.

Boland, G. J. and Hall, R. 1988c. Epidemiology of *Sclerotinia* stem rot of soybean in Ontario. *Phytopathology* 78: 1241–1245.

Boland, G. J. and Hall, R. 1994. Index of plant hosts of *Sclerotinia sclerotiorum. Can. J. Plant Pathol*. 16: 93–108.

Boland, G. J. and Hunter, J. E. 1988. Influence of *Alternaria alternata* and *Cladosporium cladosporioides* on white mould of bean caused by *Sclerotinia sclerotiorum. Can. J. Plant Pathol*. 10: 172–177.

Boland, G. J. and Inglis, G. D. 1989. Antagonism of white mold (*Sclerotinia sclerotiorum*) of bean by fungi from bean and rapeseed flowers. *Can. J. Bot*. 67: 1775–1781.

Boland, G. J. and Smith, E. A. 1991. Variation in cultural morphology and virulence among protoplast regenerated isolates of *Sclerotinia sclerotiorum. Phytopathology* 81: 766–770.

Boland, G. J., Mould, M. J. R. and Robb, J. 1993. Ultra-structures of a hypovirulent isolate of *Sclerotinia sclerotiorum* containing double–stranded RNA. *Physiol. Mol. Plant Pathol*. 16: 21–32.

Bolton, M. D., Thomma, B. P. H. J. and Nelson, B. D. 2006. Pathogen profile: *Sclerotinia sclerotiorum* (Lib.) de Bary, biology and molecular traits of a cosmopolitan pathogen. *Mol. Plant Pathol*. 7: 1–16.

Bom, M. and Boland, G. J. 2000. Evaluation of disease forecasting variables for *Sclerotinia* stem rot (*Sclerotinia sclerotiorum*) of canola. *Can. J. Plant Sci*. 80: 889–898.

Bourdot, G. W. and Harvey, I. C. 1994. A review of recent research on the microbial control of Californian thistle and other pasture weeds using the fungus *Sclerotinia sclerotiorum* as a biological herbicide. *Proc. New Zealand Grassland Assoc*. 56: 43–48.

Bourdot, G. W., Harvey, I. C., Hurrell, G. A. and Alexander, R. T. 1993. An experimental mycoherbicide utilizing *Sclerotinia sclerotiorum* controls pasture populations of *Cirsium arvense* in Canterbury. In: *Proceedings of 46th New Zealand Plant Protection Conference* (A. J. Popay, ed.). New Zealand Plant Protection Society, Rotorua, New Zealand, pp. 251–256.

Bourdot, G. W., Harvey, I. C., Hurrell, G. A. and Saville, D. J. 1995. Demographic and biomass consequences of inundative treatment of *Cirsium arvense* with *Sclerotinia sclerotiorum. Biocont. Sci. Technol*. 5: 11–25.

Bourdot, G. W., Harvey, I. C., Woodburn, T. L. (ed.), Briese, D. T. (ed.) and Corey, S. 1996. The potential of the fungus *Sclerotinia sclerotiorum* as a biological herbicide for controlling thistles in pasture. In: *Thistle Management Workshop*, Canberra, Australia, 12–13, June, 1996. *Plant Prot. Quarterly*. 11: SUP2, 259–262.

Bourdot, G. W., Hurrell, G. A. and de Jong, M. D. 2001. Risk analysis of *Sclerotinia sclerotiorum* as a mycoherbicide for pasture weed control in New Zealand. In: *Proceedings of Sclerotinia* 2001, the XI *International Sclerotinia Workshop* (C. S. Young and K. J. D. Hughes, eds.). York 8–12, July 2001, Central Science Laboratory, York, England, pp. 85–86.

Bourdot, G. W., Hurrell, G. A., Saville, D. J. and Leathwick, D. M. 2006. Impacts of applied *Sclerotinia sclerotiorum* on the dynamics of a *Cirsium arvense* population. *Weed Res*. 46: 61–72.

Bowers, R. C. 1982. Commercialization of microbial biological control agents. In: *Biological Control of Weeds with Plant Pathogens* (R. Charudattan and H. L. Walker, eds.). Wiley, New York, pp. 157–173.

Boyle, C. 1921. Studies in the physiology of parasitism. VI. Infection by *Sclerotinia libertiana. Annals Bot*. [London] 35: 337–347.

Boyetchko, S. M. 1996. Impact of soil microorganisms on weed biology and ecology. *Phytoprotect*. 77: 41–56.

Boyette, C. D., Quimby, P. C. Jr., Caesar, A. J., Birdsall, J. L., Connick, W. J. Jr., Daigle, D. J., Jackson, M. A., Egley, G. H. and Abbas, H. K. 1996. Adjuvants, formulations and spraying systems for improvement of mycoherbicides. *Weed Technol*. 10: 637–644.

Bradley, C. A., Rio, L. E. del and Johnson, B. L. 2003. First report of *Sclerotinia sclerotiorum* on niger (*Guizotia abyssinica*). *Plant Dis*. 87: 602.

Bradley, C. A., Rio, L. E. del and Chesrown, C. D. 2005. First report of soft rot caused by *Sclerotinia sclerotiorum* on borage in North Dakota. *Plant Dis*. 89: 208.

Bradley, C. A., Lamey, H. A., Endres, G. J., Henson, R. A., Hanson, B. K., McKay, K. R., Halvorson, M., Le Gare, D. G. and Porter, P. M. 2006. Efficacy of fungicides for the control of *Sclerotinia* stem rot of canola. *Plant Dis*. 90: 1129–1134.

Bremer, E., Huang, Hung Chang, Selinger, L. J., Davies, J. S. and Huang, H. C. 2000. Competence of *Coniothyrium minitans* in preventing infection of bean leaves by *Sclerotinia sclerotiorum. Plant Pathol. Bull*. 9: 69–74.

Brooks, A. H., More, W. D. and Borders, H. I. 1945. Sc1erotiniase of vegetables and tentative suggestions for its control. *Fla. Agr. Exp. Sta. Press. Bull*. 613.

Brosten, B. S. and Sands, D. C. 1986. Field trials of *Sclerotinia sclerotiorum* to control Canada thistle (*Cirsium arvense*). *Weed Sci.* 34: 377–380.

Brown, J. G. 1937. Relation of livestock to the control of sclerotinosis of lettuce. *Phytopathology* XXVII 11: 1045–1050.

Brown, J. G. and Butler, K. D. 1936. Sc1erotiniase of lettuce in Arizona. *Agric. Exp. Sta. Tech. Bull.* 63: 475–506.

Brun, H., Renard, M., Jouan, B., Tanguy, X. and Lamarque, C. 1981. Preliminary observation on some rape diseases in France: *Sclerotinia sclerotiorum, Cylindrosporium concentrium, Ramularia armoraciae. Rev. Plant Pathol.* 60: 1120 (Abstr.).

Brun, H., Jouan, B., Plessis, J. and Tribodet, M. 1983. Properties preventives et curatives de la procym-adone et du benomyl dams la lutte centre *Sclerotinia sclerotiorum* sur colza. In: *6th Rapeseed Conf.* Paris, 17–19 May, p. 187 (Abstr.).

Brun, H., Tribodet, M., Renard, M., Plessis, J. and Tanguy, X. 1987. A fluid study of rapeseed (*Brassica napus*) resistance to *Sclerotinia sclerotiorum*. In: *7th Proceedings of the International Rapeseed Conference*, pp. 1216–1221.

Buchuchanu, M. I., Karadhova, L. V. and Rotaru, F. G. 1989. Production of sunflower hybrid resistance to the main fungal diseases and broomrape - a promising means of freeing the environment of pollution. Tezisy dokladov respublikanskoi nauchnoproizvo-drtvennoi Knoferentsii. 15 iyunga, 1989. *Bel'tsy. Moldvian SSR* 39–40.

Buchwald, L. 1986. Development of a simple forecasting system for attacks of *Sclerotinia sclerotiorum* in oilseed rape in Denmark. *Reptr. Res. Centre Plant Prot. Lothenbogvij*. Lyngby, Denmark, 7 pp.

Buchwald, N. F. 1949. Studies in the *Sclerotinia*ceae 1. Taxonomy of the Sc1erotiniaceae. *K Vet. Landbohjsk. Arsskr.* 1949: 75–191.

Buchwald, N. F. 1956. On the dimorphism of the ascospores and their arrangement in the ascus of *Monilinia oxycocci* (wor.) Honey [Syn. *Sclerotinia oxycocci* wor]. *Friesia* 5: 196-203.

Buchwald, N. F. and Neergaard, P. 1973. A plea for the retention of *Sclerotinia sclerotiorum,* as type species for the *Sclerotinia* Fekl. *Emend. Friesia* 10: 96–99.

Buck, K. W. 1986. Fungal virology - an overview. In: *Fungal Virology* (K. W. Buck, ed.). CRC, Boca Raton, FL, pp. 1–84.

Buck, K. W. 1987. Viruses of plant pathogenic fungi. In: *Genetics and Plant Pathogenesis* (P. R. Day and G. J. Jellis, eds.). Blackwell Scientific, Oxford, pp. 111–126.

Budge, S. P. and Whipps, J. M. 1991. Glasshouse trials of *Coniothyrium minitans* and *Trichoderma* species for the biological control of *Sclerotinia sclerotiorum* in celery and lettuce. *Plant Pathol.* 40: 59–66.

Budge, S. P. and Whipps, J. M. 2001. Potential for integrated control of *Sclerotinia sclerotiorum* glasshouse lettuce using *Coniothyrium minitans* and reduced fungicide application. *Phytopathology* 91: 221–227.

Budge, S. P., McQuilken, M. P., Fenlon, J. S. and Whipps, J. M. 1995. Use of *Coniothyrium minitans* and *Gliocladium virens* for biological control of *Sclerotinia sclerotiorum* in glasshouse lettuce. *Biol. Control* 5: 513–522.

Bullock, S. and Willetts, H. J. 1996. Ultra-structural and histochemical studies on mycelial germination of sclerotia of *Sclerotinia minor. Mycol. Res.* 100: 561–570.

Bullock, S., Willetts, H. J. and Ashford, A. E. 1980a. The structure and histochemistry of sclerotia of *Sclerotinia minor* Jagger. I. Light and electron microscope studies on sclerotial development. *Protoplasma* 104: 315–331.

Bullock, S., Ashford, A. E. and Willetts, H. J. 1980b. The structure and histochemistry of sclerotia of *Sclerotinia minor* Jagger II. Histochemistry of extra cellular substances and cytoplasmic reserves. *Protoplasma* 104: 333–351.

Bullock, S., Adams, P. B., Willetts, H. J. and Ayers, W. A. 1986. Production of haustoria by *Sporidesmium sclerotivorum* in sclerotia of *Sclerotinia minor. Phytopathology* 76: 101–103.

Burger, O. F. 1913. Lettuce drop. *Fla. Agric. Exp. Stn. Bull.* 116: 8 pp.

Burke, D. W., Gomes, J. G. and Foeppel, W. G. 1957. Observations on *Sclerotinia* wilt of beans in Northeastern Colorado. *Plant Dis. Reptr.* 41: 72–73.

Burke, J. M. and Rieseberg, L. H. 2003. Fitness effects of transgenic disease resistance in sunflowers. *Science* 300: 1250.

Bussink, H., Buxton, F., Fraaye, B., de Graaff, L. and Visser, J. 1992. The polygalacturonase of *Aspergillus niger* are encoded by a family of diverged genes. *Eur. J. Biochem*. 208: 83–90.

Butler, G. M. 1966. Vegetative structures. In: *The Fungi - An Advanced Treatise* (G. C. Ainsworth and A. S. Sussman, eds.). Vol. II, Academic, New York, pp. 83–112.

Butzler, T. M., Bailey, J. and Beute, M. K. 1998. Integrated management of *Sclerotinia* blight in peanut: utilizing canopy morphology, mechanical pruning and fungicide timing. *Plant Dis*. 82: 1312–1318.

Buzi, A., Chilosi, G., Sillo, D. de and Magro, P. 2004. Induction of resistance in melon to *Didymella bryoniae* and *Sclerotinia sclerotiorum* by seed treatments with acibenzolar-S-methyl and methyl jasmonate but not with salicylic acid. *J. Phytopathol*. 152: 34–42.

Byrde, R. J. W. 1952. Experiments on the control of brown rot of fruits. II. Winter spraying trials. *J. Hort. Sci*. 27: 192–200.

Caesar, A. J. and Pearson, R. C. 1983. Environmental factors affecting survival of ascospores of *Sclerotinia sclerotiorum. Phytopathology* 73: 1024–1030.

Callahan, F. E. and Rowe, D. E. 1991. Use of the host-pathogen interaction system to test whether malic acid is the sole pathogenic determinant in the exudate of *Sclerotinia trifoliorum. Phytopathology* 81: 1546–1550.

Calonge, F. D. 1970. Notes on the ultra-structure of the microconidium and stroma in *Sclerotinia sclerotiorum. Arch. Mikrobiol*. 71: 191–195.

Calonge, F. D., Fielding, A. H., Byrde, R. J. W. and Akinrefon, D. A. 1969. Changes in ultrastructure following fungal invasion and the possible relevance of extra cellular enzymes. *J. Exp. Bot*. 20: 350–357.

Calotelo, M. 1974. A scanning electron microscope study of developing sclerotia of *Sclerotinia sclerotiorum. Can. J. Bot*. 52: 1127–1130.

Cameron, D. G., Hylten-Cavallius, I., Jordow, E. and Klomp, A. G. 1986. Guazatine - its use as a foliar fungicide in cereals and oilseed rape. *Br. Crop. Prot. Cent. Pest Dis*. 3: 1201–1207.

Campbell, W. A. 1947. A new species of *Coniothyrium* parasitic on sclerotia. *Mycologia* 39: 190–195.

Cannon, P.F. 1986. International Commission on the Taxonomy of Fungi (ICTF) name changes in fungi of microbiological, industrial and medical importance. Part 1, *Microbiol. Sci*. 3: 168–171.

Caprari, C., Bergmann, C., Micheli, Q., Salvi, C., Alberheim, P., Darvill, A., Cervone, F. and De Lorenzo, G. 1993. *Fusarium moniliforme* secretes four endopolygalacturonase derived from a single gene product. *Physiol. Mol. Plant Pathol*. 43: 453–462.

Carbone, I. and Kohn, L. M. 1993. Ribosomal DNA sequence divergence within internal transcribed spacer I of the *Sclerotinia*ceae. *Mycologia* 85: 415–427.

Carbone, I. and Kohn, L. M. 2001.Multilocus nested haplotype networks extended with DNA fingerprints show common origin and fine scale, ongoing genetic divergence in a wild microbial metapopulation. *Mol. Ecol*. 10: 2409–2422.

Carbone, I., Anderson, J. B. and Kohn, L. M. 1995. A group-I intron in the mitochondrial small subunit ribosomal RNA gene of *Sclerotinia sclerotiorum. Curr. Genet*. 27: 166–176.

Carbone, I., Anderson, J. B. and Kohn, L. M. 1999. Pattern of descent in clonal lineages and their multilocus finger prints are resolved with combined gene genealogies. *Evoluation* 53: 11–21.

Carisse, O. 2001. Effect of *Microsphaeropsis ochracea*, a new biological control agent on germination of sclerotia of *Sclerotinia sclerotiorum*. In: *Proceedings of Sclerotinia 2001, The XI International Sclerotinia Workshop* (C. S. Young and K. J. D. Hughes, eds.). York 8th–12th, July, 2001, Central Science Laboratory, York, England, pp. 53–54.

Carpenter, M. A., Frampton, C. and Stewart, A. 1999. Genetic variation in New Zealand populations of plant pathogen *Sclerotinia sclerotiorum. N. Z. J. Crop Hortic. Sci*. 27: 13–21.

Carpita, N. C. and Gilbeaut, D. M. 1993. Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the wall during growth. *Plant J*. 3: 1–30.

Cartia, G. and Aseri, C. 1994. The role of temperature regarding *Sclerotinia sclerotiorum* in the soil solarization method. *Acta Hort*. 366: 323–330.
Casale, W. L. and Hart, I. P. 1986. Influence of four herbicides on carpogenic germination and apothecium development of *Sclerotinia sclerotiorum. Phytopathology* 76: 980–984.
Cauahan, F. E. and Rowe, D. E. 1991. Use of a host-pathogen interaction system to test whether oxalic acid is the sole pathogenic determinant in the exudates of *Sclerotinia trifoliorum. Phytopathology* 81: 1546–1550.
Cerkauskas, R. F., Verma, P. R. and McKenzie, D. L. 1986. Effects of herbicides on *in vitro* growth and carpogenic germination of *Sclerotinia sclerotiorum. Can. J. Plant Pathol*. 8: 161–166.
Cessna, S. G., Sears, V. E., Dickman, M. B. and Low, P. S. 2000. Oxalic acid, a pathogenicity factor for *Sclerotinia sclerotiorum* suppresses the oxidative burst of the host plant. *Plant Cell* 12: 2119–2199.
Chaban, V. S., Yakubova, I. V. and Soklovskaya, M. P. 1993. New for Ukraine mycophytic fungus *Sporidesmium sclerotivorum* Uecker, Ayers et Adams on the sclerotia of *Sclerotinia sclerotiorum* (Lib.) de Bary. *Mikol. Phytopathol*. 27: 61.
Chakrabarty, P. K. 1993. Chemical management of curd rot complex of cauliflower. *Indian J. Agric. Sci*. 63: 50–55.
Chamberlain, E. E. 1932. Sclerotium disease (*Sclerotinia sclerotiorum*) of tomatoes. *N. Z. J. Agric*. 45: 260–268.
Chang, K. F., Howard, R. J., Gaudiel, R. G. and Hwang, H. F. 1997a. First report of steva as a host of *Sclerotinia sclerotiorum. Plant Dis*. 81: 311.
Chang, K. F., Howard, R. J., Gaudiel, R. G. and Hwang, R. P. 1997b. First report of *Sclerotinia sclerotiorum* on cone flower. *Plant Dis*. 81: 1093.
Charchar, M. J. D., dos Anjos, J. R. N. and Ossipi, E. 1999. Occurrence of a new disease of irrigated cotton, in Brazil caused by *Sclerotinia sclerotiorum. Pesquisa Agropecuaria Brasileira* 34: 1101–1106.
Charudattan, R. 1982. Regulation of microbial weed control agents. In: *Biological Control of Weeds with Plant Pathogens* (R. Charudattan and H. L. Walker, eds.). Wiley, New York, pp. 175–188.
Chattopadhyay, C., Meena, P. D. and Kumar, S. 2002. Management of *Sclerotinia* rot of Indian mustard using ecofriendly strategies. *J. Mycol. Plant Pathol*. 32: 194–200.
Chattopadhyay, C., Meena, P. D. and Meena, R. L. 2004. Integrated management of *Sclerotinia* rot of Indian mustard. *Indian J. Plant Prot*. 32: 88–92.
Chaudhary, B. N. 1993. Yield loss estimation by *Sclerotinia sclerotiorum* (Lib.) de Bary. *J. Inst. Agric. Ani. Sci*. 14: 113.
Chaudhary, K. C. B. and Puttoo, B. L. 1991. Seed borne nature of *Sclerotinia sclerotiorum* in safflower in India. *Indian J. Mycol. Plant Pathol*. 21: 196.
Chaves, M. S., Martinelli, J. A. and Loch, L. C. 1996a. Use of dried mycelium of *Sclerotinia sclerotiorum* as a method of inoculation and evaluation of resistance of soybean cultivars. *Summa Phytopathologica* 22: 221–224.
Chaves, M. S., Martinelli, J. A. and Loch, L. C. 1996b. Effect of root exudates of soybeans on carpogenic germination of *Whetzelinia sclerotiorum* sclerotia. *Summa Phytopathologica* 22: 256–258.
Cheah, L. H., Page, B. B. C. and Shepherd, R. 1997. Chitosan coating for inhibition of *Sclerotinia* rot of carrots. *N. Z. J. Crop Hortic. Sci*. 25: 89–92.
Chen, C. and Dickman, M. B. 2005. cAMP blocks MAPK activation and sclerotial development via Rap-1in a PKA- independent manner in *Sclerotinia sclerotiorum. Mol. Microbiol*. 55: 299–311.
Chen, C., Harel, A., Gorovoits, R., Yarden, O. and Dickman, M. B. 2004. MPAK regulation of sclerotial development in *Sclerotinia sclerotiorum* is linked with pH and cAMP sensing. *Mol. Plant Microbe. Interact*. 17: 404–413.
Chen, D. Y. and Wu, W. S. 1990. Effect of high temperature on the survival of *Sclerotinia sclerotiorum* and its application. *Plant Prot. Bull*. [Taiwan] 32: 277–288.
Chen, W., Schatz, B., Henson, B., Mc Phee, K. E. and Muehlbauer, F. J. 2006. First report of *Sclerotinia* stem rot of chickpea caused by *Sclerotinia sclerotiorum* in North Dakota and Washington. *Plant Dis*. 90: 114.

Chen, Y. and Wang, D. 2005. Two convenient methods to evaluate soybean for resistance to *Sclerotinia sclerotiorum. Plant Dis*. 89: 1268–1272.

Chenz, G. Z. M. and Jiao, H. S. 1994. Studies on predicting the epidemic of soybean stem rot caused by *Sclerotinia sclerotiorum. Soybean Sci*. 13: 48–52.

Chet, I. and Henis, Y. 1975. Sclerotial morphogenesis in fungi. *Ann. Rev. Phytopathol.* 13: 169–192.

Christiansen, M. A. 1966. Variation in apothecial structures of some *Sclerotinia*ceae matured under natural and artificial conditions. Thesis. Cornell University. Ithaca, New York 77 p.

Christias, C. and Lockwood, J. L. 1973. Conservation of mycelial constituents in four sclerotium forming fungi in nutrient deprived conditions. *Phytopathology* 63: 602–605.

Chun, D., Kao, L. B., Lockwood, J. L. and Isleib, T. G. 1987. Laboratory and field assessment of resistance in soybean to stem rot caused by of *Sclerotinia sclerotiorum. Plant Dis*. 71: 811–815.

Cilliers, A. J. and Wyk, P. S. van. 1999. Preliminary observations on resistance of groundnuts to *Sclerotinia* stem blight. *Afr. Plant Prot*. 5: 73–75.

Ciray, E. and Findlater, W. T. 1960. *Sclerotinia sclerotiorum* on peas in Kineardineshire. *Plant Pathol.* 9: 130–132.

Citola, M., Wymore, L. A. and Watson, A. K. 1991. *Sclerotinia*, a potential mycoherbicide for lawns. *Weed Sci. Soc. Am. Abstracts* 31: 81 (Abstr.) 242.

Clark, M. F. and Adams, A. N. 1977. Characteristics of the micro plate method of enzyme linked immunosorbent assay for viruses. *J. General Virol*. 34: 475–483.

Clarkson, J. P., Staveley, J., Phelps, K., Young, C. S. and Whipps, J. M. 2003. Ascospore release and survival in *Sclerotinia sclerotiorum. Mycol. Res*. 107: 213–222.

Clarkson, J. P., Phelps, K., Whipps, J. M., Young, C. S., Smith, J. A. and Watling, M. 2004. Forecasting *Sclerotinia* disease on lettuce: toward developing a prediction model for carpogenic germination of sclerotia. *Phytopathology* 94: 268–279.

Cline, M. N. and Jacobsen, B. J. 1983. Methods for evaluating soybean cultivars for resistance to *Sclerotinia sclerotiorum. Plant Dis*. 67: 784–786.

Cobb, P. R., Powell, N. L. and Porter, D. M. 1977. Survey of *Sclerotinia sclerotiorum* blight disease losses in peanut field by remote sensing. *Proc. Am. Peanut Res. Edu. Assoc*. 9: 94.

Codron, P. D. 1974. Ultra-structural study of some aspects of the ascus development of *Sclerotinia sclerotiorum* (Lib.) de Bary. *Annales des Sciences Naturelles, Botanique, Paris* 15: 255–276.

Coffelt, T. A. and Porter, D. M. 1980. Screening peanut (*Arachis hypogaea* L.) for resistance to *Sclerotinia* blight. *Proc. Am. Peanut Res. Edu. Soc*. 12.

Coffelt, T. A. and Porter, D. M. 1982. Screening peanut for resistance to *Sclerotinia* blight. *Plant Dis*. 66: 385–387.

Coffelt, T. A., Porter, D. M. and Garren, K. H. 1980. Breeding for resistance to *Cylindrocladium* black rot and *Sclerotinia* blight in peanuts. In: *Proceedings of the Peanut Breeding Symposium North Carolina State University* July, 1980, pp. 44–48.

Coffelt, T. A., Porter, D. M. and Mozingo, R. W. 1982. Registration of Virginia. 81, bunch pea nut. *Crop Sci*. 22: 1085–1086.

Coley-Smith, J. R. and Cooke, R. C. 1971. Survival and germination of fungal sclerotia. *Annu. Rev. Phytopath*. 9: 65–92.

Colotelo, N. 1973. Physiological and biochemical properties of the exudates associated with developing sclerotia of *Sclerotinia sclerotiorum* (Lib.) de Bary. *Can. J. Microbiol*. 19: 73–79.

Colotelo, N. 1974. A scanning electron microscope study of developing sclerotia of *Sclerotinia sclerotiorum. Can. J. Bot*. 52: 1127–1130.

Cook, G. E. 1973. Some aspects of the epidemiology of white mold on dry beans caused by *Whetzelinia sclerotiorum* in Western Nebraska. Ph.D. thesis, University of Nebraska, Lincon, 81 p.

Cook, G. E., Steadman, J. R. and Boosalis, M. G. 1975. Survival of *Whetzelinia sclerotiorum* and initial infection of dry edible beans in Western Nebraska. *Phytopathology* 65: 250–255.

Corato, U. de 1996. Research on stem rot of kenaf caused by *Sclerotinia sclerotiorum* in Basilicata. *Informatore Fitopatologico* 46: 30–32.

Corato, U. de and Baviello, G. 2000. Occurrence of *Sclerotinia sclerotiorum* on *Brassica carinata* in Southern Italy (*Basilicata*). *Informatore Fitopatologico* 50: 61–63.

Corsini, D. L. and Le Tourneau, D. 1973. Organic acid metabolism in *Sclerotinia sclerotiorum. Arch. Mikrobiol*. 90: 59–64.

Costa, G. R., Costa, J. L. and da, S. 2004. Effect of fungicide application in the soil on the carpogenic and myceliogenic germination of *Sclerotinia sclerotiorum. Pesquisa Agropecuaria Trop*. 34: 133–138.

Cother, E. J. 2000. Pathogenicity of *Sclerotinia sclerotiorum* to *Chrysanthemoides monilifera* ssp. *rotundata* (bitou bush) and selected species of the coastal flora in eastern Australia. *Biol. Control* 18: 10–17.

Cother, E. J., Nikandrow, A. and Gilbert, R. L. 1996. *Sclerotinia sclerotiorum*, a potential biocontrol agent for *Chrysanthemoides monilifera* (bitoubush). In: *Proceedings of IX International Symposium on Biological Control of Weeds, 1996. Stellenbosch, South Africa* (V. V. Moran and J. H. Hoffman, eds.). University of Capetown, South Africa, pp. 529–530.

Cotton, P., Rascle, C. and Fevre, M. 2002. Characterization of PG2, an early endoPG produced by *Sclerotinia sclerotiorum* expressed in yeast. *FEMS Microbiol. Lett*. 213: 239–244.

Cotton, P., Kasza, Z., Bruel, C., Rascle, C. and Fevre, M. 2003. Ambient pH controls the expression of endogalacturonase genes in the necrotrophic fungus *Sclerotinia sclerotiorum. FEMS Microbiol. Lett*. 277: 163–169.

Couper, G. 2001. The biology, epidemiology and control of *Sclerotinia sclerotiorum* on carrots in North East Scotland. Ph.D. thesis, University of Aberdeen, Aberdeen, Scotland.

Coyne, D. P., Steadman, J. R. and Anderson, F. N. 1974. Effect of modified plant architecture of great northern dry bean varieties (*Phaseolus vulgaris*) on white mold severity and components of yield. *Plant Dis. Reptr*. 58: 379–382.

Coyne, D. P., Steadman, J. R. and Magnuson, S. 1976. Breeding for white mold disease resistance and avoidance due to ideo type in beans. *Annl. Rep. Bean Improv. Coop*. 19: 21–23.

Coyne, D. P., Steadman, J. R. and Gehwar, H. F. 1977a. Genetic variation, inheritance and breeding strategy for resistance to *Sclerotinia sclerotiorum* in beans (*Phaseolus vulgaris* L.). *Rep. Bean. Improv. Coop. Net. Dry Bean Council. Biennial Conf*. November, 8–10, 1977, Emeryville, CA, pp. 19–21.

Coyne, D. P., Steadman, J. R. and Schwartz, H. F. 1977b. Reaction of *Phaseolus* dry bean germination to *Sclerotinia sclerotiorum. Plant Dis. Reptr*. 61: 226–230.

Coyne, D. P., Steadman, J. R. and Schwartz, H. F. 1978. Effects of genetic blends of dry bean (*Phaseolus vulgaris*) of different plant architecture on apothecia production of *Sclerotinia sclerotiorum* and white mould infection. *Euphytica* 27: 225–231.

Crisan, A. 1964. Researches on chemical means of *Sclerotinia sclerotiorum* (Lib.) de Bary. on sunflower. *Rev. Appl. Mycol*. 43: 1376 (Abstr.).

Crisan, A., Hodisan, V. and Pop, M. 1978. Investigation on the possibility of using volatile oils from plants in the prevention and control of some fungus diseases in stored vegetables and fruits. *Contributii Botanice Univ. Babes – Bolyai din Cluj Napoca.* Cluj Napoca, Romania. 269–275.

Cruickshank, I. A. M. and Perrin, D. R. 1971. Studies on phytoalexins. XI. The induction, antimicrobial spectrum and chemical assay of phaseollin. *Phytopathol. Z.* 70: 209–229.

Cruickshank, R. H. 1983. Distinction between *Sclerotinia* species by their pectic zygograms. *Trans. Br. Mycol. Soc*. 80: 117–119.

Cruickshank, A. W., Cooper, M. and Ryley, M. J. 2002. Peanut resistance to *Sclerotinia minor* and *S. sclerotiorum. Aus. J. Agric. Res*. 53: 1105–1110.

Cubeta, M. A., Cody, B. R., Kohli, Y. and Kohn, L. M. 1997. Clonality in *Sclerotinia sclerotiorum* on infected cabbage in eastern North Carolina. *Phytopathology* 87: 1000–1004.

Cubeta, M. A., Cody, B. R., Hudyncia, J. and Shew, H. D. 1998a. Ecology and population biology of *Sclerotinia sclerotiorum* on cabbage in eastern North Carolina. In: *Proceedings of 10th International Sclerotinia Workshop* (B. D. Nelson and T. J. Gulya, eds.). BSPP and ISPP, Fargo, ND, pp. 32–33.

Cubeta, M. A., Cody, B. R., Hudyncia, J., Nelson, B. D. (ed.) and Gulya, T. J. 1998b. Evaluation of fungicides, an antioxidant and a plant activator for managing *Sclerotinia* head rot of cabbage.

In: *Proceedings of 1998 International Sclerotinia Workshop*, Fargo, ND, September 9–12, 1998, pp. 51–52.
Dabkeviciene, G. and Dabkevicus, Z. 2005. Evaluation of wild red clover (*Trifolium pratense* L.) ecotypes and hybrid populations (*Trifolium pratense* L. x *Trifolium diffusum* Ehrh.) for clover rot resistance (*Sclerotinia trifoliorum* Erikss.). *Biologija* 2005(3): 54–58.
Daniel, J. T., Templeton, G. E., Smith, R. J. Jr. and Fox, W. T. 1973. Biological control of northern joint vetch in rice with an endemic fungal disease. *Weed Sci*. 21: 303–307.
Danielson, G. A., Nelson, B. D. and Helms, T. C. 2004. Effect of *Sclerotinia* stem rot on yield of soybean inoculated at different growth stages. *Plant Dis*. 88: 297–300.
Dann, E., Diers, B., Byrum, J. and Hammerschmidt, R. 1998. Effect of treating soybean with 2, 6-dichloroisonicotinic acid (INA) and benzothiadiazole (BTH) on seed yields and the level of disease caused by *Sclerotinia sclerotiorum* in field and greenhouse studies. *Eur. J. Plant Pathol*. 104: 271–278.
Das, M. G., Das, B. C. and Sarmah, D. K. 2002. *In vitro* studies of some antagonists against *Sclerotinia sclerotiorum* (Lib) de Bary. *J. Agric. Sci. Soc. North East India* 15: 67–70.
Davet, P. and Martin, C. 1980. Development in the control of *Sclerotinia* on lettuce. *Bull. Tech. des. P.O*. 96: 82–89.
Davies, J. M. L., Gladders, P., Young, C., Dyer, C., Hiron, L., Locke, T., Lockley, D., Ottway, C., Smith, J., Thorpe, G. and Watling, M. 1999. Petal culturing to forecast *Sclerotinia* stem rot in winter oilseed rape: 1993–1998. *Aspects Appl. Biol*. 56: 129–134.
Davies, P. and Muncey, M. 2004. Prothioconazole for control of *Sclerotinia sclerotiorum* in oilseed rape/canola. *Pflanzenschutz Nachrichten Bayer* 57: 283–293.
Davis, K. R., Darvill, A. G., Albersheim, P. and Dell, A. 1986. Host pathogen interactions XXIX-Oligogalacturonides released from sodium polypectate by endopolygalact-uronic acid lyase are elicitors of phytoalexin in soybean. *Plant Physiol*. 80: 568–577.
Debnam, J.R. and Smith, I.M. 1976. Changes in the isoflavones and pterocarpans of red clover on infection with *Sclerotinia trifoliorum* and *Botrytis cinerea*. *Physiol. Plant Pathol*. 9: 9–23.
de Bary, A. 1886. Ueber einige Sclerotinien and Sclerotienkrankheiten. *Bot. Zeitung* 44: 377–474.
de Bary, A. 1887. Comparative Morphology and Biology of Fungi. *Mycetozoa* and *Bacteria*. London/New York/Oxford, Clerenden, 525 pp.
Degener, J., Melchinger, A. E. and Hahn, V. 1999. Optimal allocation of resources in evaluating current sunflower inbred lines for resistance to *Sclerotinia. Plant Breed*. 118: 157–160.
Delclos, B. and Raynal, G. 1995. Comparison of techniques for the production of *Sclerotinia trifoliorum* ascospores in the laboratory for forage legumes resistance tests. *J. Phytopath*. 143: 345–348.
De Lorenzo, G., D’ Ovidio, R. and Cervone, F. 2001. The role of polygalacturonase-inhibiting proteins (PGIPs) in defense against pathogenic fungi. *Annu. Rev. Phytopathol*. 39: 313–335.
del Rio, L. E., Martinson, C. A. and Yang, X. B. 2002. Biological control of *Sclerotinia* stem rot of soybean with *Sporidesmium sclerotivorum. Plant Dis*. 86: 999–1004.
del Rio, L. E., Venette, J. R. and Lamey, H. A. 2004. Impact of white mold incidence on dry bean yield under non-irrigated conditions. *Plant Dis*. 88: 1352–1356.
Dennis, R.W.G. 1956. A revision of the British *Helotiaceae* in the herbarium of the Royal Botanic Garden, Kew, with notes of related European species. *Mycol. Pap*. 62: 1–206.
Dennis, R. W. G. 1978. *British Ascomycetes*. Vol. xxvi, Cramer, Vaduz, 585 p.
De Silva, A., Bolton, M. D. and Nelson, B. D. 2005. Transformation of *Sclerotinia sclerotiorum* with the green fluorescent protein gene and expression of fluorescence of host tissues. *Phytopathology* 95: 523.
Dhawan, S. 1980. Production of protease by *Sclerotinia sclerotiorum* cause of white rot of *Brassica juncea. Curr. Sci*. 49: 361–362.
Dhawan, S. and Srivastava, S. K. 1987. Trans-eliminative (lytic) pectic enzymes in *Sclerotinia* infection of *Brassica juncea. Indian J. Mycol. Plant Pathol*. 17: 325–327.
Diamantopoulou, A., Litkei, J., Skopa, C. and Christias, C. 2000. Effects of inhibitors of sclerotium formation on the sclerotial mycoparasite *Coniothyrium minitans* and its host *Sclerotinia sclerotiorum. Mycol. Res*. 104: 1449–1452.

Dias, B. B. A., Cunha, W. G., Morais, L. S., Vianna, G. R., Rech, E. L., Capdeville, G. de and Aragao, F. J. L. 2006. Expression of an oxalate decarboxylase gene from *Flammulina* sp. in transgenic lettuce (*Lactuca sativa*) plants and resistance to *Sclerotinia sclerotiorum. Plant Pathol*. 55: 187–193.

Dickman, M. B. and Mitra, A. 1992. *Arabidopsis thaliana* as a model for studying *Sclerotinia sclerotiorum* pathogenesis. *Physiol. Mol. Plant Pathol*. 41: 255–263.

Dickman, M. B., Park, Y. K., Oltersdorf, T., Li, W., Clemente, T. and French, R. 2001. Abrogation of disease development in plants expressing animal antiapoptotic genes. *Proc. Natl. Acad. Sci. USA* 98: 6957–6962.

Dickson, J. G. 1930. Studies on *Sclerotinia Sclerotiorum* (Lib.) de Bary. Ph.D. Thesis, Cornell University, Ithaca, New York, 136 pp.

Dickson, J. G. 1956. *Diseases of Field Crops*. McGraw-Hill, India, p. 517.

Dickson, M. H., Petzoldt, R., Dras, J. S., Crute, I. and Monteiro, A. A. 1996. Breeding for resistance to *Sclerotinia sclerotiorum* in *Brassica oleracea. Acta Hort*. 407: 103–108.

Diers, B.W., Kopisch Obuch, F. J., Hoffman, D. D., Hartman, G. L., Pedersen, W. L., Grau, C.R. and Wang, D. 2006. Registration of AxN-1-55 soybean germplasm with partial resistance to *Sclerotinia* stem rot. *Crop Sci*. 46: 1403–1404.

Dillard, H. R. and Grogan, R. G. 1985. Relationship between sclerotial spatial pattern and density of *Sclerotinia minor* and the incidence of lettuce drop. *Phytopathology* 75: 90–94.

Dillard, H. R. and Hunter, J. E. 1986. Association of common ragweed with *Sclerotinia* rot of cabbage in New York State. *Plant Dis*. 70: 26–28.

Dillard, H. R., Cobb, A. C. and Bozard, B. T. 1991. Velvet leaf, a new host for *Sclerotinia sclerotiorum. Plant Dis*. 75: 863.

Dillard, H. R., Ludwig, J. W. and Hunter, J. E. 1995. Conditioning sclerotia of *Sclerotinia sclerotiorum* for carpogenic germination. *Plant Dis*. 79: 411–415.

Dillon-Weston, W. A. R., Loveless, R. A. and Taylor, E. R. 1946. Clover rot. *J. Agric. Sci*. 36: 18–28.

Dimopoulou, L. 1975. Influence de quelques substances in vitro sur la formation des sclerotes du *Sclerotinia sclerotiorum. Ann. De I Institut. Phytopathol. Benaki*. 11: 127–131.

Diniz, S. P. S. S., Utumi, H., Bonzanini, F. and Bueno, M. S. 2005. Bioactivity of medicinal plants in the control of isolated *Sclerotinia* of *Stevia rebaudiana* (Bert.) Bertoni. *Revista Brasileira de Plantas Medicinais*. 7: 22–25.

Dittmer, U. and Weltzien, H. C. 1990. A rapid viability test for sclerotia with fluorescein diacetate. *J. Phytopathol*. 130: 59–64.

Dixon, G. R. 1975. Resistance of red and white clover cultivars to clover rot (*Sclerotinia trifoliorum). Ann. Appl. Biol*. 81: 276–278.

Dixon, G. R. and Doodson, J. K. 1974. Techniques for testing the resistance of red clover cultivars to *Sclerotinia trifoliorum* Erikss. (Clover rot). *Euphytica* 23: 671–679.

Djikstra, J. 1964. Inoculation with ascospores of *Sclerotinia trifoliorum* for detection of clover rot resistance red clover. *Euphytica* 13: 314–329.

Djikstra, J. 1966. Rotting by spread of mycelium form ascospore lesions of *Sclerotinia trifoliorum. Neth. J. Plant Pathol*. 72: 279–283.

Dohroo, N. P. 1988. Germplasm reaction of cauliflower to stalk rot (*Sclerotinia sclerotiorum*). *Indian J. Plant Pathol*. 6: 144.

Domsch, K. H., Gams, W. and Anderson, T. 1980. *Compendium of Soil Fungi*. Vol. I, Academic Press, New York, pp. 712–716.

Donaldson, P. A., Anderson, T., Lane, B. G., Davidson, A. L. and Simmonds, D. H. 2001. Soybean plants expressing an active oligomeric oxalate oxidase from the wheat gf-2-8 (germin) gene are resistant to the oxalate secreting pathogen *Sclerotinia sclerotiorum. Physiol. Mol. Plant Pathol*. 59: 297–307.

Dorrell, D. G. and Huang, R. C. 1978. Influence of *Sclerotinia* wilt on seed yield and quality of sunflower wilted at different stages of development. *Crop Sci*. 18: 274–276.

Dos Santos, A. F. and Dhingra, O. D. 1982. Pathogenicity of *Trichoderma* spp. on the sclerotia of *Sclerotinia sclerotiorum. Can. J. Bot*. 60: 472–475.

Dow, R. L. and Lumsden, R. D. 1975. Histology of resistance of *Phaseolus coccineus* (Scarlet runner bean) to *Sclerotinia sclerotiorum. Proc. Am. Phytopathol. Soc*. 2: 122 (Abstr.).

Dow, R. L., Porter, D. M. and Powell, N. L. 1988a. Effect of environmental factors on *Sclerotinia minor* and *Sclerotinia* blight of peanut. *Phytopathology* 78: 672–676.

Dow, R. L., Powell, N. L. and Porter, D. M. 1988b. Effect of modification of the plant canopy environment on *Sclerotinia* blight of peanut. *Peanut Sci*. 15: 1–5.

Drozdova, D. L. 1988. Source material of red clover for breeding for resistance to diseases in the north-west region of the *R.S.F.S.R. Sbornik Nauchnykh Trudov po Prikladnoi Botanike, Genetike i selektsii*. 120: 67–70.

Duczek, L. J. and Morrall, R. A. A. 1971. *Sclerotinia* in Saskatchewan in 1970. *Can Plant Sur*. 51: 116–121.

Dudka, M., Langton, S., Shuler, R., Kurle, J., Grau, C. R., Robert, P. C. (ed.), Rust, R. H. (ed.) and Larson, W. E. 1999. Use of digital imagery to evaluate disease incidence and yield loss caused by *Sclerotinia* stem rot of soybeans. In: *Proceedings of the Fourth International Conference on Precision Agriculture*, St. Paul, MN, July 19–22, 1998, Part A and Part B, pp. 1549–1558.

Dueck, J. 1977. *Sclerotinia* in rapeseed. *Canada Agriculture* 22: 7–9.

Dueck, J. 1979. Selecting for resistance to *Sclerotinia* in Sunflower. *Can. Agric*. 24: 11.

Dueck, J., Morrall, R. A. A. and McKenzie, D. L. 1983. Control of *Sclerotinia sclerotiorum* in rapeseed with fungicides. *Can. J. Plant Pathol*. 5: 289–293.

Dumont, K. P. 1971. *Sclerotinia*ceae. 11. Lambertelia Mem. *N.Y. Bot. Gard*. 22: 1–178.

Dumont, K. P. and Korf, R. P. 1971. *Sclerotinia*ceae 1. Genetic nomenculature. *Mycologia* 63: 157–168.

Duniway, J. M., Abawi, G. S. and Steadman, J. R. 1977. Influence of soil moisture on the production of apothecia by sclerotia of *Whetzelinia sclerotiorum. Proc. Am. Phytopathol. Soc*. 4: 115 (Abstr.).

Dutton, M. V. and Evans, C. S. 1996. Oxalate production by fungi: its role in pathogenicity and ecology in the soil environment. *Can. J. Microbiol*. 42: 881–895.

Echandi, E. and Walker, J. C. 1957. Pectolytic enzymes produced by *Sclerotinia sclerotiorum. Phytopathology* 47: 303–306.

Eken, C., Demirci, E., Esitken, A. E. and Ercisli, S. 2003. First report of orchid (*Orchis laxiflora*) as a host of *Sclerotinia minor*, discovered in Turkey. *Plant Pathol*. 52: 802.

Eken, C., Ercisli, S., Esitken, A., Demirci, E. and Yuen, G. Y. 2005. First report of crown and stem rot of orchid (*Orchis palustris*) caused by *Sclerotinia minor. Plant Dis*. 89: 913.

Ekins, M. G., Aitken, E. A. B. and Goulter, K. C. 2002. Carpogenic germination of *Sclerotinia minor* and potential distribution in Australia. *Australas. Plant Pathol*. 31: 259–265.

Ekins, M. G., Aitken, E. A. B. and Goulter, K. C. 2005. Identification of *Sclerotinia* species. *Australas. Plant Pathol*. 34: 549–555.

El Ghaouth, A. 1994. Manipulation of defense system with elicitors to control post harvest diseases. In: *Biological Control of Post-harvest Diseases - Theory and Practice* (C. L. Wilson and M. E. Wisniewski, eds.). CRC, Boca Raton, FL, pp. 153–167.

El Tarabily, K. A., Soliman, M. H., Nassar, A. H., Al Hassani, H. A., Sivasithamparam, K., Mc Kenna, F. and Hardy, G. E. St. J. 2000. Biological control of *Sclerotinia minor* using a chitinolytic bacterium and actinomycetes. *Plant Pathol*. 49: 573–583.

Elad, Y. 1992. The use of antioxidants (free radical scavengers) to control grey mould (*Botrytis cinerea*) and white mould (*Sclerotinia sclerotiorum*) in various crops. *Plant Pathol*. 41: 417–426.

Elad, Y. 2000. Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. *Crop Prot*. 19: 709–714.

Elgin, J. H. and Beyer, E. H. 1968. Evaluation of selected alfalfa clover for resistance to *Sclerotinia trifoliorum* Erikss. *Crop Sci*. 8: 265–266.

Ellis, M. B. and Waller, J. M. 1998. *Sclerotinia fuckeliana*, IMI Descriptions of Fungi and Bacteria. (44), Sheet 481, CABI Bioscience, Bakeham Lane, Egham, Surrey, TW20 9TY.

Elliston, J. E. 1982. Hypovirulence. In: *Advances in Plant Pathology* (D. S. Ingram and P. H. Williams, eds.). Vol. l., Academic, Toronto, Canada, pp. 1–33.

Ericksson, J. 1880. Om Klofverrotan med sarskildt afseende pa dess upptradante I vart land under aren 1878–79. *Kgl. Landtbruks Akad. Handl. Tidskr*. 19: 28–42.

Erneberg, M., Strandberg, B. and Jensen, B. D. 2003. Susceptibility of a plant invader to a pathogenic fungus: an experimental study of *Heracleum mantegazzianum* (Giant Hogweed) and *Sclerotinia sclerotiorum*. In: *Plant Invasions: Ecological Threats and Management Solutions*, pp. 355–372.

Errampolli, D. and Kohn, L. M. 1996. Electrophoretic karyotypes of *Sclerotinia sclerotiorum. Appl. Environ. Microbiol*. 62: 4247–4251.

Ervio, L. R., Halkilabti, A. M. and Pohjakallio, O. 1964. The survival in the soil of sclerotia of two *Sclerotinia* species and their ability to form mycelia. *Adv. Front. Plant Sci*. 8: 121–133.

Escande, A. R., Laich, F. S. and Pedraza, M. V. 2002. Field testing of honeybee dispersed *Trichoderma* spp. to manage sunflower head rot (*Sclerotinia sclerotiorum*). *Plant Pathol*. 51: 346–351.

Eva, B. 2003. Biological control of the pathogen *Sclerotinia sclerotiorum* (Lib.) de Bary from soybean (*Glycine hispida* (Moench.) Maxim) crops. *Cercetari Agron. Moldova* 36: 66–72.

Eva, B. and Andrei, E. 2000. The effect of *Sclerotinia sclerotiorum* (Lib.) de Bary attack on the morpho-physiological traits in sunflower. *Probleme de Protectia Plantelor* 28: 47–67.

Expert, J. M. and Digat, B. 1995. Biocontrol of *Sclerotinia* wilt of sunflower by *Pseudomonas fluorescens* and *Pseudomonas putida* strains. *Can. J. Microbiol*. 41: 685–691.

Farady, L. 1988. Effect of the treatment date and fungicide combination on sunflower infection with *Phomopsis helianthi* Munt-cvet *et al* and *Sclerotinia sclerotiorum* Lib (de Bary). *Novenyvedelem* 24: 441–444.

Farr, D. F., Bills, G. F., Chamuris, G. P. and Rossman, A. Y. 1989. *Fungi on Plants and Plant Products in the United States*. APS, St. Paul, MN, 1252 p.

Favaron, F., Alghisi, P., Marciano, P. and Magro, P. 1988. Polygalacturonase isoenzymes and oxalic acid production by *Sclerotinia sclerotiorum* in soybean hypocotyls as elicitors of glycolin. *Physiol. Mol. Plant Pathol*. 33: 385–395.

Favaron, F., D' Ovidio, R., Porceddu, E. and Alghisi, P. 1994. Purification and molecular characterization of a soybean polygalacturonase inhibiting protein. *Planta* 195: 80–87.

Favaron, F., Sella, L. and D'Ovidio, R. 2004. Relationships among endopolygalacturonase, oxalate, pH and plant polygalacturonase inhibiting protein (PGIP) in the interaction between *Sclerotinia sclerotiorum* and soybean. *Mol. Plant Microbe Interact*. 17: 1402–1409.

Fedorinchik, N. S. and Vanderflaas, L. K. 1958. Effect of antagonistic activity of the soil fungi *Trichoderma lignorum* Harz. on increase in yield of agricultural crops. *Rev. Appl. Mycol*. 37: 24 (Abstr.).

Feng, X. and Thaning, Christian. 2001. Suppression of apothecial formation in *Sclerotinia sclerotiorum* by bacteria. In: *Proceedings of Sclerotinia 2001. The XI International Sclerotinia Workshop* (C. S. Young and K. J. D. Hughes, eds.). York 8–12 July 2001, Central Science Laboratory, York, England, pp. 149–150.

Fernando, W. G. D., Nakkeeran, S. and Zhang, Yi Lan. 2004. Ecofriendly methods in combating *Sclerotinia sclerotiorum* (Lib.) de Bary. In: *Recent Research Developments in Environmental Biology*. Vol. 1, Part II, pp. 329–347.

Ferrar, P. H. and Walker, J. R. L. 1993. A-Diphenol oxidase inhibition, an additional role for oxalic acid in the phytopathogenic arsenal of *Sclerotinia sclerotiorum* and *Sclerotium rolfsii. Physiol. Mol. Plant Pathol*. 43: 415–422.

Finck, O. 1989. Application of transformation products of calcium cyanamide in the field for inhibition of sclerotial germination of *Sclerotinia sclerotiorum*, casual agent of stem rot of rape. *Zeitsehrift fur Pflanzenkrankheiten and Pflanzenschutz*. 96: 508–520.

Finck, G. and Bomer, H. 1985. The effect of transformation products of calcium cyanamide on the sclerotial germination of *Sclerotinia sclerotiorum*, the fungus causing rape cancer. *J. Plant Dis. Prot*. 92: 449–454.

Finlayson, J. E., Rimmer, S. R. and Pritchard, M. K. 1989. Infection of carrots by *Sclerotinia sclerotiorum. Can. J. Plant Pathol*. 11: 242–246.

Fischer, H. 1979. Control of rots of head lettuce. *Gesunde. Pflanzen*. 31: 125–131.

Fitzgerald, A., van Kan, J. A. L. and Plummer, K. M. 2004. Simultaneous silencing of multiple genes in the apple scab fungus *Venturia inaequalis* by expression of RNA with chimeric inverted repeats. *Fungal Genet. Biol*. 41: 963–971.

Fogliata, G. M., Ramallo, N. V. de and Ploper, L. D. 1999. Shoot wilt in lemons caused by *Sclerotinia sclerotiorum* (*Sclerotinia* twig blight). *Advance Agroindustrial*. 20: 28–30.

Ford, E. J., Miller, R. V., Gray, H. and Sherwood, J. E. 1995. Heterokaryon formation and vegetative compatibility in *Sclerotinia sclerotiorum. Mycol. Res*. 99: 241–247.

Fraissinet-Tachet, L. and Fevre, M. 1996. Regulation by galacturonic acid of pectinolytic enzyme production by *Sclerotinia sclerotiorum. Curr. Microbiol*. 33: 49–53.

Fraissinet-Tachet, L., Reymond-Cotton, P. and Fevre, M. 1995. Characterization of a multi-gene family encoding an endopolygalacturonase in *Sclerotinia sclerotiorum. Curr. Microbiol*. 29: 96–100.

Fraissinet-Tachet, L., Reymond Cotton, P. and Fevre, M. 1996. Molecular karyotype of the phytopathogenic fungus *Sclerotinia sclerotiorum. Curr. Genet*. 29: 496–501.

Fravel, D. R. 1998. Use of *Sporidesmium sclerotivorum* for biocontrol of sclerotial plant pathogens. In: *Plant Microbe Interactions and Biological Control* (G. J. Boland and L. D. Kuykendall, eds.). Marcel Dekker, New York, pp. 37–48.

Fravel, D. R., Adams, P. B. and Potts, W. E. 1992. Use of disease progress curves to study the effects of the biocontrol agents *Sporidesmium sclerotivorum* on lettuce drop. *Biocont. Sci. Technol*. 2: 341.

Fravel, D. R., Connick, W. J., Jr., Grimm, C. C. and Lloyd, S. W. 2002. Volatile compounds emitted by sclerotia of *Sclerotinia minor*, *Sclerotinia sclerotiorum*, and *Sclerotium rolfsii. J. Agric. Food Chem*. 50: 3761–3764.

Frandsen, K.J. 1946. Studies over *Sclerotinia trifoliorum* Erikss. *Danske Forlag* 222p. Copenhagen.

Freeman, J., Calderon, C., Ward, E. and Mc Cartney, H. A. 2001. Polymerase chain reaction (PCR) based assays for the detection of inoculum of *Sclerotinia* spp. In: *Proceedings of Sclerotinia 2001*, *the XI International Sclerotinia Workshop* (C. S. Young and K. J. D. Hughes, eds.). York 8–12 July 2001, Central Science Laboratory, York, England, pp. 127–128.

Freeman, J., Ward, E., Calderon, C. and Mc Cartney, A. A. 2002. Polymerase chain reaction (PCR) assay for the detection of inoculum of *Sclerotinia sclerotiorum. Eur. J. Plant Pathol*. 108: 877–886.

Freeman, T. E., Charudattan, R. and Conway, K. E. 1978. Status of the use of plant pathogens in the biological control of weeds. In: *Proceedings of the IV International Symposium on the Biological Control of Weeds, 1976* (T. E. Freeman, ed.). Institute of Food and Agricultural, University of Florida, Gainesville, FL, pp. 201–206.

Fu, S., Lu, Z., Chen, Y., Gi, C., Pu, H. and Zhang, J. 1990. Inheritance of apetalous character in *Brassica napus* L and its potential in breeding. *Proceedings of the Symposium on China International Rapeseed Sciences*, 7–8.

Fu, Y. L. and Tang, J. X. 1994. A new technique to control *Sclerotinia* rot of rape (*Sclerotinia sclerotiorum*). *Bull. Agric. Sci. Tech*. 3: 30.

Fuckel, L. 1870. Symbolic mycologicae. Beitrage zur Kenntniss der Rheinischen Pilze. I *Jhrb. Nassauischen Vercins Naturk*. 23: 1–459.

Fuhlbohm, M. J., Tatnell, J. R. and Ryley, M. J. 2003. First report of stem rot and wilt of chickpea caused by *Sclerotinia minor* in Queensland, Australia. *Australas. Plant Pathol*. 32: 323–324.

Gabrielson, R. L., Guilford, R. K. and Coahran, D. R. 1971. Field control of white and grey mold of beans in western Washington. *Plant Dis. Reptr*. 55: 234–238.

Gabrielson, R. L., Anderson, W. C. and Nyvall, R. F. 1973. Control of *Sclerotinia sclerotiorum* in cabbage seed yield with aerial applications of benomyl and ground application of cyanamide. *Plant Dis. Reptr*. 57: 164–166.

Gaetan, S. and Madia, M. 1995. *Sclerotinia minor* Jagger, a pathogen of French bean and Russian tarragon. *Boletin-de- Sanidad Vegetal, Plagas*. 21: 611–615.

Gaetan, S., Madia, M. and Reyna, S. 1997. Aromatic umbelliferae, hosts of *Sclerotinia sclerotiorum* (Lib.) de Bary. *Boletin de Sanidad Vegetal, Plagas*. 23: 377–383.

Gaffney, T., Friedrich, L., Vernooij, B., Negrotto, D., Nye, G., Uknes, S., Ward, E., Kessman, H. and Ryals, J. 1993. Requirements of salicylic acid for the induction of systemic acquired resistance. *Science* 261: 754–756.

Gao, S., Choi, G. H., Shain, L. and Nuss, D. L. 1996. Cloning and targeted disruption of *enpg-1*, encoding the major *in vitro* extracellular endopolygalacturonase of the chestnut blight fungus *Cryphonectria parasitica. Appl. Environ. Microbiol*. 62: 1984–1990.

Garcia-Maceira, F. I., Di Pietro, A., Huertas-Gonzalez, M. D., Ruiz-Roldan, M. C. and Roncero, M. I. G. 2001. Molecular characterization of an endopolygalacturonase from *Fusarium oxysporum* expressed during early stages of infection. *Appl. Environ. Microbiol*. 67: 2191–2196.

Garibaldi, A., Minuto, A., Gilardi, G. and Gullino, M. L. 2001. First report of *Sclerotinia sclerotiorum* on *Gazania* sp. hybrid in Italy. *Plant Dis*. 85: 1207.

Garibaldi, A., Minuto, A., Pensa, P., Bruzzone, C., Salvi, D. and Gilardi, G. 2002a. *Sclerotinia sclerotiorum* agent of basal rot of Gazania, Campanula and Schizantus. *Colture Protette* 31: 105–107.

Garibaldi, A., Minuto, A. and Gullino, M. L. 2002b. First report of *Sclerotinia sclerotiorum* on *Campanula carpatica* and *Schizanthus* and *Wisetonensis* in Italy. *Plant Dis*. 86: 71.

Garibaldi, A., Minuto, A. and Gullino, M. L. 2003. First report of white mold caused by *Sclerotinia sclerotiorum* on Persian buttercup (*Ranunculus asiaticus*) in Italy. *Plant Dis*. 87: 1151.

Garibaldi, A., Minuto, A. and Gullino, M. L. 2004. First report of white mold caused by *Sclerotinia sclerotiorum* on blue marguerite (*Felicia amelloides*) in Italy. *Plant Dis*. 88: 575.

Garibaldi, A., Minuto, A. and Gullino, M. L. 2005. First report of *Sclerotinia* stem rot and watery soft rot caused by *Sclerotinia sclerotiorum* on sand rocket (*Diplotaxis tenuifolia*) in Italy. *Plant Dis*. 89: 1241.

Garrod, B. and Lewis, B. G. 1982. The effect of falcarindiol on hyphal growth of *Mycocentrospora acerina. Trans. Br. Mycol. Soc*. 75: 166–169.

Geary, J. R. 1978. Host-parasite interactions between the cultivated carrot (*Daucus carota* L.) and *Sclerotinia sclerotiorum* (Lib.) de Bary. Ph.D. thesis, University of East Anglia, East Anglia.

Geeson, J. D., Browne, K. M. and Everson, H. P. 1988. Storage disease of carrots in East Anglia 1978–82 and the effects of some pre- and post-harvest factors. *Ann. Appl. Biol*. 112: 503–514.

Gepp, V., Silvera, E., Casanova, S. and Tricot, D. 2001. Solarization in the management of lettuce drop (*Sclerotinia* spp.). In: *Proceedings of Sclerotinia 2001, The XI International Sclerotinia Workshop* (C. S. Young and K. J. D. Hughes, eds.). York 8–12 July 2001, Central Science Laboratory, York, England, pp. 135–136.

Gerlagh, M., Kruse, M., Goossen-van deGeijn, H. M. and Whipps, J. M. 1994. Growth and survival of *Coniothyrium minitans* on lettuce leaves in contact with soil in the presence and absence of *Sclerotinia sclerotiorum. Eur. J. Plant Pathol*. 100: 55–59.

Gerlagh, M., Goossen- van de Geijn, H. M., Hoogland, A. E., Verdam, B. and Fokkema, N. J. 1995a. Biological control of *Sclerotinia sclerotiorum* as a strategic choice. *Gewasbescherming* 26: 158–161.

Gerlagh, M., Goossen-van de Geijn, H. M., Verdam, B. and Fokkema, N. J. 1995b. Biological control of white mould (*Sclerotinia sclerotiorum*) in various crops by application of *Coniothyrium minitans*. In: *Biological Control of Sclerotium Forming Fungi* (L. M. Whipps and T. Gerlagh, eds.). *IOBC/WPRS Bull*. 18: 13.

Gerlagh, M., Goossen-van de Geijn, H. M., Fokkema, N. J. and Vereijken, P. F. G. 1999. Long term biosanitation by application of *Coniothyrium minitans* on *Sclerotinia sclerotiorum* infected crops. *Phytopathology* 89: 141–147.

Gerlagh, M., Goossen- van de Geijn, H. M., Hoogland, A. E. and Vereijken, P. F. G. 2003. Quantitative aspects of infection of *Sclerotinia sclerotiorum* sclerotia by *Coniothyrium minitans*-timing of application, concentration and quality of conidial suspension of the mycoparasite. *Eur. J. Plant Pathol*. 109: 489–502.

Ghabrial, S. A. 1980. Effects of fungal viruses on their hosts. *Ann. Rev. Phytopathol*. 18: 44–61.

Ghaffar, A. 1972. Some observation on the parasitism of *Coniothyrium minitans* on the sclerotia of *Sclerotinia sclerotiorum. Pak. J. Bot*. 4: 85–87.

Ghasolia, R. P. and Shivpuri, A. 2004. Neem - a new host of *Sclerotinia sclerotiorum. J. Mycol. Plant Pathol*. 34: 200–201.

Ghasolia, R. P. and Shivpuri, A. 2005. Screening of rapeseed-mustard genotypes for resistance against *Sclerotinia* rot. *Indian Phytopath*. 58: 242.

Ghasolia, R. P. and Shivpuri, A. 2007. Morphological and pathogenic variability in rapeseed and mustard isolates of *Sclerotinia sclerotiorum.Indian Phytopath*. 60: 76–81.

Ghasolia, R. P., Shivpuri, A. and Bhargava, A. K. 2004a. Some common weed species as hosts for *Sclerotinia sclerotiorum. J. Mycol. Plant Pathol*. 34: 670–671.

Ghasolia, R. P., Shivpuri, A. and Bhargava, A. K. 2004b. *Sclerotinia* rot of Indian mustard in Rajasthan. *Indian Phytopath*. 57: 76–79.

Gilbert, A. H. and Bennett, C. W. 1917. *Sclerotinia trifoliorum*, the cause of stem rot of clovers and alfalfa. *Phytopathology* 7: 432–442.

Gilbert, R. G. 1987. Crown and stem rot of alfalfa caused by *Sclerotinia sclerotiorum. Plant Dis*. 71: 739–742.

Gilbert, R. G. 1991. Burning to reduce sclerotia of *Sclerotinia sclerotiorum* in alfalfa seed field of Southeastern Washington. *Plant Dis*. 75: 141–142.

Gindrat, D., Frei, P., Vullioud, P. and Pellet, D. 2003. Prevision du risque de sclerotiniose du colza en Suisse. *Rev. Suisse Agric*. 35: 225–231.

Godoy, G., Steadman, J. R., Dickman, M. B. and Dam, R. 1990. Use of mutants to demonstrate the role of oxalic acid in pathogenicity of *Sclerotinia sclerotiorum* on *Phaseolus vulgaris. Physiol. Mol. Plant Pathol*. 37: 179–191.

Gonzalez, T. G., Henderson, D. M. and Koike, S. T. 1998. First report of bell pepper (*Capsicum annuum*) as a host of *Sclerotinia minor* in California. *Plant Dis*. 82: 832.

Gonzalez, V., Ramallo, N. V. de, Daniel-Ploper, L., Cecilia, Ghio, A., Galvez, R. and Vizgarra, O. 2003. Detection of *Sclerotinia minor* Jagger in lentils (*Lens culinaris*) and chickpea (*Cicer arietinum*) in the province of Tucuman, Argentina. *Advance Agroindustrial* 24: 39–41.

Goodman, D. M. and Burpee, L. L. 1991. Biocontrol of dollar spot disease of creeping bentgrass. *Phytopathology* 81: 1438–1446.

Gossan, B. D., Rimmer, S. R. and Holley, J. D. 2001. First report of resistance to benomyl fungicide in *Sclerotinia sclerotiorum. Plant Dis*. 85: 1206.

Gotoechan, H.M. 1999. Effets de deux is oculants endomycorhiziens commerciaaux sor le rendement et la qualite de la carotte (*Daucus carota* L.) en champ et sur le developement de deux maladies en entrepot. M.Sc. Thesis Laval University, Quebec, Canada.

Govrin, E. M. and Levine, A. 2000. The hypersensitive response facilitates plant infection by the necrotrophic pathogen *Botrytis cinerea. Curr. Biol*. 10: 751–757.

Gracia-Garza, J. A., Bailey, B. A., Paulitz, T. C., Lumsden, D. P., Reeleder, R. D. and Roberts, D. P. 1997a. Effect of sclerotial damage of *Sclerotinia sclerotiorum* on the mycoparasitic activity of *Trichoderma harzianum. Biocontrol Sci. Technol*. 7: 401–413.

Gracia-Garza, J. A., Reeleder, R. D. and Poulitz, T. C. 1997b. Degradation of sclerotia of *Sclerotinia sclerotiorum* by fungus gnats *(Bradysia coprophila)* and the biocontrol fungi *Trichoderma* spp. *Soil Biol. Biochem*. 29: 123–129.

Gracia-Garza, J. A., Neumann, S., Vyn, T. J. and Boland, G. J. 2002. Influence of crop rotation and tillage on production of apothecia by *Sclerotinia sclerotiorum. Can. J. Plant Pathol*. 24: 137–143.

Graf, F. and Schumacher, T. 1995. *Sclerotinia glacialis* sp. nv. from the alpine zone of Switzerland. *Mycol. Res*. 99: 113–117.

Graham, J. H. and Hanson, R. G. 1953. Field inoculation of red clover with *Sclerotinia trifoliorum. Plant Dis. Reptr*. 37: 518–520.

Granata, G. and Tirro, A. 1989. Serious damage by *Sclerotinia sclerotiorum* (Lib.) Massee on globe artichoke (*Cynara scolymus* L). *Difesa delle Piante* 12: 127–131.

Grau, C. R. 1988. *Sclerotinia* stem rot of soybean. In: *Soybean diseases of the North Central Region*, (Wyllie, T. D. and Scott, D. H., (eds), American Phytopathological Soc. St Paul, MN., pp 56–66

Grau, C. R. and Bissonnette, H. L. 1974. *Whetzelinia* stem rot of soybean in Minnesota. *Plant Dis. Reptr*. 58: 693–695.

Grau, C. R. and Radke, V. L. 1984. Effects of cultivars and cultural practices on *Sclerotinia* stem rot of soybean. *Plant Dis*. 68: 56–58.

Grau, C. R., Radke, V. L. and Gillespie, F. L. 1982. Resistance of soybean cultivars to *Sclerotinia sclerotiorum. Plant Dis*. 66: 506–508.

Grauert, P., Schlosser, E. and Schuster, W. 1980. Investigation on the possibility of varieties and lines of sunflower *(Helianthus annuus* L) to *Sclerotinia sclerotiorum* (Lib.) de Bary. *Angewandte Botanik*. 54: 349–364.

Gray, E. G. and Findlater, W. I. 1960. *Sclerotinia sclerotiorum* on peas in Kineardineshire. *Plant Pathol*. 9: 130–132.

Green, S., Gaunt, R. E., Harvey, I. C. and Bourdot, G. W. 1998. Histopathology of *Ranunculus acris* infected by a mycoherbicide, *Sclerotinia sclerotiorum. Australas. Plant Pathol*. 27: 73–79.

Grill, D. 1979. *Botrytis* and *Sclerotinia* on lettuce. *Revue Horticole*. 198: 47–48 (Fr.).

Grison, R., Grezes-Besset, B., Schneider, M., Lucante, N., Olsen, L., Leguay, J. J. and Toppan, A. 1996. Field tolerance to fungal pathogens of *Brassica napus* constitutively expressing a cimeric chitinase gene. *Nature Biotech*. 14: 643–646.

Grogan, R. G. 1979. *Sclerotinia* species: summary and comments on needed research. *Phytopathology* 69: 908–910.

Grogan, R. G. and Abawi, G. S. 1975. Influence of water potential on growth and survival of *Whetzelinia sclerotiorum. Phytopathology* 65: 122–138.

Groves, J. W. and Bowerman, G. A. 1955. *Sclerotinia borealis* in Canada. *Can. J. Bot*. 33: 591–594.

Guan, C. Y., Li, F. Q., Li, X., Chen, S. Y., Wang, G. H. and Liu, Z. S. 2003. Resistance of the double-low rapeseed cultivar Xiangyou 15 (*Brassica napus*) to *Sclerotinia sclerotiorum. Acta Agronomica Sinica* 29: 715–718.

Guenin, M. C. De. 1991. Towards Sc1erotinia control on Sunflower. *Phytoma*. 428: 27–28.

Guimaraes, R. L. and Stotz, H. U. 2004. Oxalate production by *Sclerotinia sclerotiorum* deregulates guard cells during infection. *Plant Physiol*. 136: 3703–3711.

Gugel, R. K. and Morrall, R. A. A. 1986. Inoculum disease relationship in Sc1erotinia stem rot of rapeseed in Saskatchewan. *Can. J. Plant Pathol*. 8: 89–96.

Gullino, M. L., Minuto, G., Mocioni, M. and Garibaldi, A. 1996. Reduction of dosages of methyl bromide for soil disinfection in Italy. *Medodelingen Faculteit Landbouwkundige in Toegepaste Brologische Wetenschappen Universitiet Gent*. 61: 547–553.

Gullino, M. L., Minuto, A., Minuto, G. and Garibaldi, A. 1998. Chemical and physical alternatives to methyl bromide and their combination in the control of *Rhizoctonia solani* and *Sclerotinia sclerotiorum* in the open field. *Brighton Crop Prot. Conf.: Pests & Diseases-1998*: Vol. 2: *Proceedings of the International Conference*, November 16–19, 1998. Brighton, pp. 693–700.

Gupta, R., Awasthi, R. P. and Kolte, S. J. 2004a. Effect of nitrogen and sulphur on incidence of *Sclerotinia* rot of mustard. *Indian Phytopath*. 57: 193–194.

Gupta, R., Awasthi, R. P. and Kolte, S. J. 2004b. Influence of sowing dates on the incidence of *Sclerotinia* stem rot of rapeseed-mustard. *Ann. Plant Prot. Sci*. 12: 223–224.

Gupta, S. K. and Agarwala, R. K. 1988. Biological control of *Sc1erotinia* stalk rot of cauliflower. *Indian J. Plant Pathol*. 6: 71–74.

Gupta, S. K., Agarwala, R. K. and Sharma, S. L. 1987. Effect of crop rotation on management of stalk rot of cauliflower. *Indian J. Plant Pathol*. 5: 79–80.

Gupta, S. K., Shyam, K. R. and Dohroo, N. P. 1997. *Sclerotinia* wilt of french bean. *Indian Phytopath*. 50: 593.

Haas, J. H. and Bolwyn, B. 1972, Ecology and epidemiology of *Sc1erotinia* wilt of white beans in Ontario. *Can. J. Plant. Sci*. 52: 525–533.

Haas, J. H. and Bolwyn, B. 1974. Predicting and controlling white mold epidemics in white beans. *Can. Agric*. 18: 28–29.

Hahn, V., Micic, Z., Melchinger, A. E. and Bauer, E. 2001. QTL - analysis of *Sclerotinia sclerotiorum* resistance in sunflower. In: *Proceedings of Sclerotinia 2001, The XI International Sclerotinia Workshop* (C. S. Young and K. J. D. Hughes, eds.). York 8–12 July 2001, Central Science Laboratory, York, England, pp. 57–58.

Hall, B. H., Mc Mahon, R. L. and Wicks, T. J. 2002. First report of *Sclerotinia sclerotiorum* on grape (*Vitis vinifera*) in South Australia. *Australas. Plant Pathol*. 31: 417–418.

Hallock, D. L. and Porter, D. M. 1979. Nutrient effects on *Sc1erotinia* blight disease in peanuts. *Proc. Am. Peanut Res. Educ. Soc*. 11: 62.

Hallock, D. L. and Porter, D. M. 1981. Effect of applied plant nutrients on *Sclerotinia minor* blight incidence in peanuts (*Arachis hypogaea*). *Peanut Sci*. 8: 48.

Hambleton, S., Walker, C. and Kohn, L. M. 2002. Clonal lineages of *Sclerotinia sclerotiorum* previously known from other crops predominate in 1999–2000 samples from Ontario and Quebec soybean. *Can. J. Plant Pathol*. 24: 309–315.

Hammann, T., Kohler, H. and Friedt, W. 1994. Inter-specific hybridization as a basis in sunflower breeding (*Helianthus annuus* L) for disease resistance. *Vortrage fur Ptlanzenzuchtung* 30: 151–157.

Hampel, M., Locher, F. and Saur, R. 1981. Control of *Sclerotinia sclerotiorum* and *Botrytis cinerea* on rape and sunflower with Vinclozolin. *Proc. Br. Crop Prot. Conf. Pests Dis*. 2: 521–527.

Hancock, J. G. 1966. Degradation of pectic substances associated with pathogenesis by *Sclerotinia sclerotiorum* in sunflower and tomato stems. *Phytopathology* 56: 975–979.

Hancock, J. G. 1967. Hemicellulose degradation in sunflower hypocotyls infected with *Sclerotinia sclerotiorum. Phytopathology* 57: 203–206.

Hancock, J. G. 1972. Changes in cell membrane permeability in sunflower hypocotyls infected with *Sclerotinia sclerotiorum. Plant Physiol*. 49: 358–364.

Handoro, F., Sandhu, K. S., Singh, R. S. and Singh, P. P. 2002. Biological control of white rot (*Sclerotinia sclerotiorum*) of pea (*Pisum sativum* L.). *J. Res. Punjab Agric. Univ*. 39: 382–390.

Hannusch, D. J. and Boland, G. J. 1996. Influence of air temperature and relative humidity on biological control of white mold of bean (*Sclerotinia sclerotiorum*). *Phytopathology* 86: 156–162.

Hansen, J. M., Tobias, D. J., Balbyshev, N. F., Stack, R. W. and Lee, C. W. 2001. Effect of preharvest benomyl spray on winter storage of carrots. *Phytopathology* 91: 5178 (Abstr.).

Hao, J. J. and Subbarao, K. V. 2005. Comparative analysis of lettuce drop epidemics caused by *Sclerotinia minor* and *Sclerotinia sclerotiorum. Plant Dis*. 89: 717–725.

Hao, J. J. and Subbarao, K. V. 2006. Dynamics of lettuce drop incidence and *Sclerotinia minor* inoculum under varied crop rotations. *Plant Dis*. 90: 269–278.

Hao, J. J., Subbarao, K. V. and Koike, S. T. 2003. Effect of broccoli rotation on lettuce drop caused by *Sclerotinia minor* and on the population density of sclerotia in soil. *Plant Dis*. 87: 159–166.

Hara, K. and Yanagita, Y. 1967. Using calcium cyanamide to control rape *Sclerotinia* rot (Japanese). *Proc. Kyushu Assoc. Plant Prot*. 13: 13.

Harel, A., Gorovits, R. and Yarden, O. 2005. Changes in protein kinase A activity accompany sclerotial development in *Sclerotinia sclerotiorum. Phytopathology* 95: 397–404.

Harel, A., Bercovich, S. and Yarden, O. 2006. Calcineurin is required for sclerotial development and pathogenicity of *Sclerotinia sclerotiorum* in an oxalic acid independent manner. *Mol. Plant Microbe Interact*. 19: 682–693.

Hargitay, L. 1982. The susceptibility of different varieties of sunflower to *Sclerotinia sclerotiorum* (Lib.) de Bary. *Novenyvedelem*. 19: 359.

Harikrishnan, R. and del Rio, L. E. 2006. Influence of temperature, relative humidity, ascospore concentration and length of drying of colonized dry bean flowers on white mold development. *Plant Dis*. 90: 946–950.

Harrison, J. G. and Hargreaves, A. J. 1977. Production and germination of *Botrytis fabae* microconidia. *Trans. Brit. Mycol. Soc*. 69: 332–335.

Harter, L.L. and Zaumeyer, W.J. 1944. A monographic study of bean diseases and methods for their control. *Tech. Bull. U.S. Dep. Agric*. 868: pp 160

Hartill, W. F. T. 1980. Aerobiology of *Sclerotinia sclerotiorum* and *Botrytis cinerea* spores in New Zealand tobacco crops. *N. Z. J. Agric. Res*. 23: 259–262.

Hartill, W. F. T. and Campbell, J. M. 1973. Control of *Sclerotinia* in tobacco seed beds. *Plant Dis. Reptr*. 57: 932–934.

Hartill, W. F. T. and Underhill, A. P. 1976. Puffing in *Sclerotinia sclerotiorum* and *S. minor. N. Z. J. Bot*. 14: 355–358.

Hartman, C. L., Albaugh, D. A., Secok, G. A. and Venette, J. R. 1987. *In vitro* screening of dry bean calli to identify resistance to *Sclerotinia sclerotiorum. Phytopathology* 77: 1773 (Abstr.).

Hartman, G. L., Kull, L. and Huang, Y. H. 1998. Occurrence of *Sclerotinia sclerotiorum* in soybean fields in east-central Illinois and enumeration of inocula in soybean seed lots. *Plant Dis*. 82: 560–564.

Hartman, G. L., Gardner, M. E., Hymowitz, T. and Naidoo, G. C. 2000. Evaluation of perennial *Glycine* species for resistance to soybean fungal pathogens that cause *Sclerotinia* stem rot and sudden death syndrome. *Crop Sci*. 40: 545–549.

Harvey, I. C., Alexander, R. A., Waipara, N. W. and Savile, D. J. 1994. The effect of inoculum substrate and dew period on the pathogenicity of *Sclerotinia sclerotiorum* when applied as a mycoherbicide to California thistle (*Cirsium arvense*). *Aust. Plant Pathol*. 23: 50–56.

Harvey, I. C., Foley, L. M. and Saville, D. T. 1995. Survival and germination of shallow buried sclerotia of *Sclerotinia sclerotiorum* in pastures in Canterbury. *N. Z. J. Agric. Res*. 38: 279–284.

Harvey, I. C., Bourdot, G. W. and Zydenbos, S. M. 2001. Giant buttercup (*Ranunculus acris* L.) control in dairy pasture using a mycoherbicide based on *Sclerotinia sclerotiorum. New Zealand Plant Protection Proceedings of the Conference Palmerston North, New Zealand*. Vol. 54, August 14–16, 2001, pp. 120–124.

Hasan, S. 1988. Biocontrol of weeds with microbes. In: *Biocontrol of Plant Diseases* (K. G. Mukerji and K. L. Garg, eds.). Vol. 1. CRC, Boca Raton, FL, pp. 129–151.

Hau, F. C., Beute, M. K. and Smith, T. 1982, Effect of soil pH and volatile stimulants from remoistened peanut leaves on germination of sclerotia of *Sclerotinia minor. Plant Dis*. 66: 223–224.

ten Have, A., Mulder, W., Visser, J. and van Kan, J. A. 1998. The endopolygalacturonase gene *Bcpg-1* is required for full virulence of *Botrytis cinerea. Mol. Plant Microbe Interact*. 11: 1009–1016.

Hawthorne, B. T. 1973. Production of apothecia of *Sclerotinia minor. N. Z. J. Agric Res*. 16: 559–560.

Hawthorne, B. T. 1974. *Sclerotinia minor* on lettuce: effect of plant growth on susceptibility to infection. *N. Z. J. Agric. Res*. 17: 387–392.

Hawthorne, B. T. 1975. Effect of mulching on the incidence of *Sclerotinia minor* on lettuce. *N. Z. J. Exp. Agric*. 3: 273–274.

Hawthorne, B. T. 1976. Observations on the development of apothecia of *Sclerotinia minor* Jagg. in the field. *N. Z. J. Agric. Res*. 19: 383–386.

Hawthorne, B. T. and Jarvis, W. R. 1973. Differential activity of fungicides on various stages in the life cycle of *Sclerotinia* spp. *N. Z. J. Agric. Res*. 16: 551–557.

Hegedus, D. D. and Rimmer, S. R. 2005. *Sclerotinia sclerotiorum*: when “to be or not to be” a pathogen? *FEMS Microbiol. Lett*. 251: 177–184.

He, Kun Yan, Yi, Bin, Fu, Ting Dong and Tu, Jin Xing. 2005. Genetic analysis of resistance to *Sclerotinia sclerotiorum* in *Brassica napus* L. *Acta Agron. Sinica*. 31: 1495–1499.

Held, V.M. and Haenseler, C.M. 1953. Cross inoculation with New Jersey isolates of *Sclerotinia sclerotiorum, S. minor* and *S. trifoliorum*. *Plant Dis. Reptr*. 37: 515–517.

Held, Y. M. 1955. Physiological differences between a normal and a degenerate strain of *Sclerotinia trifoliorum. Phytopathology* 45: 39–42.

Hemery-Tardin, M. C. 1990. Markers of *Sclerotinia* tolerance for breeding sunflower phenotic markers. *Bull. CETIOM*. 106: 6–7.

Hemery-Tardin, M. C., Labrouhe, D. T. de, Jay, M., Ledoigt, G. and Vear, F. 1998. Effect of infection by *Sclerotinia* spp. on the phenolic metabolism of sunflower capitula and leaves. *Helia* 21: 19–32.

Henderson, R. M. 1962a. An inhibitory growth correlation in the apothecial stipe of *Sclerotinia sclerotiorum. Nature* 195: 826.

Henderson, R. M. 1962b. Some aspects of the life cycle of the plant pathogen *Sclerotinia sclerotiorum* in Western Australia. *J. Royal Soc. Western Aust*. 45: 133–135.

Henis, Y. and Chet, I. 1975. Microbiological control of plant pathogens: In: *Advances in Applied Microbiology* (D. Perlman, ed.). Vol. 19, pp. 85–111.

Henn, H. J., Steiner, V., Wingender, R. and Schnable, H. 1997. Wild type sunflower clones: source for resistance against *Sclerotinia sclerotiorum* (Lib.) de Bary stem infection. *J. Appl. Bot. Angewandte Botanik*. 71: 5–9.

Henson, J. M., Butler, M. J. and Day, A. W. 1999. The dark side of mycelium; melanins of phytopathogenic fungi. *Annu. Rev. Phytopathol*. 37: 447–471.

Hieu, N.T. 2007 Integrated management of *Sclerotinia* rot (*Sclerotinia sclerotiorum* (Lib.) de Bary) of Indian mustard (*Brassica juncea* L.) Czern & Coss. M. Sc. Thesis submitted to the Department of Plant Pathology, CCS HAU, Hisar. pp 135.

Higgins, B. B. 1927. Physiology and parasitism of *Sclerotium rolfsii* (Sacc.). *Phytopathology* 17: 417–448.

Hillman, B. I., Fulbright, D. W., Nuss, D. L. and van Alfen, N. K. 1995. Hypoviridae, *Virus Taxonomy* In: *Sixth Report Int. Comm. Taxonomy of Viruses* (F. A. Murphy, C. M. Fauquet, D. H. L. Bishap, S. A. Ghabrial, A. W. Jarvis, G. P. Martelli, M. A. Mayo, and M. D. Summers, eds.). Springer, New York, pp. 261–264.

Hind-Lanoiselet, T. L., Lanoiselet, V. M., Lewington, F. K., Ash, G. J. and Murray, G. M. 2005. Survival of *Sclerotinia* sclerotia under fire. *Australas. Plant Pathol*. 34: 311–317.

Hirst, J. M. 1959. Spore liberation and dispersal. In: *Plant Pathology Problem and Progress. 1908–1958* (S. Holton et al., eds.). Wisconsin University Press, Madison, WI, pp. 529–538.

Hoadley, A. D. 1963. Control of carrot storage disease organisms with sodium orthophenlphenate. *Plant Dis. Reptr*. 47: 900–903.

Hoes, H. C. 1977. Importance of *Coniothyrium minitans* in survival of sclerotia of *Sclerotinia sclerotiorum* in wilted sunflower. *Can. J. Bot*. 55: 289.

Hoes, J. A. 1971. *Sclerotinia* root rot of sunflower. In: *Proceedings of the Manitoba Agronomy Conference*, Winnipeg, Canada, December, 14–15, pp. 66.

Hoes, J. A. and Huang, H. C. 1975. *Sclerotinia sclerotiorum*: viability and separation of sclerotia from soil. *Phytopathology* 65: 1431–1432.

Hoes, J. A. and Huang, H. C. 1976. Control of *Sclerotinia* basal stem rot of sunflower: a progress report. In: *Proceedings of the Sunflower Forum*. Fargo, ND, p. 18.

Hoffman, D. D., Diers, B. W., Hartman, G. L., Nickell, C. D., Nelson, R. L., Pedersen, W. L., Cober, E. R., Graef, G. L., Steadman, J. R., Grau, C. R., Nelson, B. D., Rio, L. E. del, Helms, T., Anderson, T., Poysa, V., Rajcan, I., Stienstra, W. C. and del Rio, L. E. 2002. Selected soybean plant introductions with partial resistance to *Sclerotinia sclerotiorum. Plant Dis*. 86: 971–980.

Holst-Jensen, A., Kohn, L. M., Jakobsen, K. S. and Schumacher, T. 1997a. Molecular phylogeny and evolution of *Monilinia* (*Sclerotinia*ceae) based on coding and non-coding rDNA sequences. *Am. J. Bot*. 84: 686–701.

Holst-Jensen, A., Kohn, L. M. and Schmacher, T. 1997b. Nuclear rDNA phylogeny of the *sclerotinia*ceae. *Mycologia* 89: 885–899.

Holst-Jensen, A., Vaage, M. and Schumacher, T. 1998. An approximation to the phylogeny of *Sclerotinia* and related genera. *Nordic J. Bot*. 18: 705–719.

Holcomb, G. E. 1990. *Apios americana* and *Trillium foetidissimum* as new host of *Sclerotinia sclerotiorum. Plant Dis*. 74: 720.

Holcomb, G. E. 2001. First report of occurrence of *Sclerotinia* blight on petunia in Louisiana. *Plant Dis*. 85: 1, 95.

Holcomb, G. E. 2005. First report of *Sclerotinia* stem rot and death of *Osteospermum* spp. hybrid cultivars caused by *Sclerotinia sclerotiorum* in Louisiana. *Plant Dis*. 89: 911.

Hollowell, J. E. and Shew, B. B. 2001. Yellow nutsedge (*Cyperus esculentus* L.) as a host of *Sclerotinia minor. Plant Dis*. 85: 562.

Hollowell, J. E. and Shew, B. B. 2004. First report of stem and leaf blight caused by *Sclerotinia minor* on *Geranium carolinianum* in North Carolina. *Plant Dis*. 88: 312.

Hollowell, J. E. and Shew, B. B. 2005a. First report of *Sclerotinia minor* on *Allium vineale* in North Carolina. *Plant Dis*. 89: 908.

Hollowell, J. E. and Shew, B. B. 2005b. First report of *Sclerotinia minor* on *Sida spinosa* in North Carolina. *Plant Dis*. 89: 1128.

Hollowell, J. E., Shew, B. B., Cubeta, M. A. and Wilcut, J. W. 2003. Weed species as hosts of *Sclerotinia minor* in peanut fields. *Plant Dis*. 87: 197–199.

Honda, Y. H. and Yunoki, T. 1977. Control of *Sclerotinia* disease of greenhouse eggplant and cucumber by inhibition of development of apothecia. *Plant Dis. Reptr*. 61: 1036–1040.

Honda, Y. H. and Yunoki, T. 1980. Inhibition of fungal sporulation by ultraviolet absorbing vinyl film and its application to disease control. *JARG* 14: 79–83.

Honey, E. E. 1928. The monilioid species of *Sclerotinia. Mycologia*. 20: 127–157.
Hoover, R. J. and Kucharek, T. A. 1995. First report of a leaf spot on perennial peanut caused by *Sclerotinia homoeocarpa. Plant Dis*. 79: 1249.
Horie, H. and Hoshi, H. 2002. First report of *Sclerotinia* rot of hybrid bouvardia in Japan. *Annu. Report Kanto Tosan Plant Prot. Soc*. 49: 69–71.
Howard, R. J. and Valent, B. 1996. Breaking and entering host penetration by fungal rice blast pathogen *Magnaporthe grisea. Annu. Rev. Microbiol*. 50: 491–512.
Hsiang, T., Yang, Lin and Barton, Wayne. 1998. Relative virulence of isolates of *Sclerotinia sclerotiorum* to propiconazole. *Eur. J. Plant Pathol*. 104: 163–169.
http://www.broad.mit.edu/annotation/fungi/*sclerotinia*/sclerotiorum;
http://www.caes.uga.edu;
http://www.ces.ncsu.edu;
http://www.potatodiseases.org;
http://www.turfgrassmanagement.psu.edu;
http://www.whitemoldresearch.com
Hu, X., Bidney, D. L., Yalpani, N., Duvick, J. P., Crasta, O., Folkerts, O. and Lu, G. 2003. Over expression of a gene encoding hydrogen-peroxide generating oxalate oxidase evokes defense responses in sunflower. *Plant Physiol*. 133: 170–181.
Hua, Z. F., Zhang, X. W., Ren, X. Y. and Wang, C. B. 1987. Occurrence of and damage by *Sclerotinia sclerotiorum* in sunflowers. *Oil Crops China* 2: 51–54.
Hua, Z. F., Liu, X. M., Li, Y., Li, H., Zhang, G. J., Zhang, J. H., Wang, C. B. and Lu, Y. X. 1994. Study on integrated control of *Sclerotinia sclerotiorum* of sunflower in Jilin Province. *Acta Phytophylacica Sinica* 21: 127–134.
Huang, H. C. 1976. Biological control of *Sclerotinia* wilt in sunflower. *Rep. Annu. Conf. Manit. Agron*. 69–72.
Huang, H. C. 1977. Importance of *Coniothyrium minitans* in survival of sclerotia of *Sclerotinia sclerotiorum* in wilted sunflowers. *Can. J. Bot*. 55: 289–295.
Huang, H. C. 1978. *Gliocladium catenulatum:* hyperparasite of *Sclerotinia sclerotiorum* and *Fusarium* species. *Can. J. Bot*. 56: 2243–2246.
Huang, H. C. 1979. Biological control of *Sclerotinia* wilt of sunflower. *Can. Agric*. 24: 12.
Huang, H. C. 1980. Control of *Sclerotinia* wilt of sunflower by hyperparasites. *Can. J. Plant Pathol*. 2: 26–32.
Huang, H. C. 1981. Tan sclerotia of *Sclerotinia sclerotiorum. Can. J. Plant Pathol*. 3: 136–138.
Huang, H. C. 1982. Morphologically abnormal sclerotia of *Sclerotinia sclerotiorum. Can. J. Microbiol*. 28: 87–91.
Huang, H. C. 1983. Histology, amino acid leakage and chemical composition of normal and abnormal sclerotia of *Sclerotinia sclerotiorum. Can. J. Bot*. 61: 1443–1447.
Huang, H. C. 1991. Induction of myce1iogenic germination of sclerotia of *Sclerotinia sclerotiorum* by exposure to sub-freezing temperatures. *Plant Pathol*. 40: 621–625.
Huang, H. C. and Blackshaw, R. E. 1995. Influence of herbicides on the carpogenic germination of *Sclerotinia sclerotiorum* sclerotia. *Bot. Bull. Acad. Sinica* 36: 59–64.
Huang, H. C. and Dorrell, D. G. 1978. Screening sunflower seedling for resistance to toxic metabolites produced by *Sclerotinia sclerotiorum. Can. J. Plant Sci*. 58: 1107–1110.
Huang, H. C. and Dueck, J. 1980. Wilt of sunflower from infection by mycelial germinating sclerotia of *Sclerotinia sclerotiorum. Can. J. Plant Pathol*. 2: 47–52.
Huang, H. C. and Erickson, R. S. 2002. Overwintering of *Coniothyrium minitans*, a mycoparasite of *Sclerotinia sclerotiorum*, on the Canadian Prairies. *Australas. Plant Pathol*. 31: 291–293.
Huang, H. C. and Erickson, R. S. 2004. Effect of soil treatment of fungal agents on control of apothecia of *Sclerotinia sclerotiorum* in canola and safflower fields. *Plant Pathol. Bull*. 13: 1–6.
Huang, H. C. and Hoes, J. A. 1976. Penetration and infection of *Sclerotinia sclerotiorum* by *Coniothyrium minitans. Can. J. Bot*. 54: 406–410.

Huang, H. C. and Hoes, J. A. 1980. Importance of plant spacing and sclerotial position to development of *Sclerotinia* wilt of sunflower. *Plant Dis*. 64: 81–84.

Huang, H. C. and Kemp, G. A. 1989. Growth habit of dry beans (*Phaseolus vulgaris*) and incidence of white mold (*Sclerotinia sclerotiorum*). *Plant Prot. Bull*. [Taiwan] 31: 304–309.

Huang, H. C. and Kokko, E. G. 1987. Ultra-structure of hyperparasitism of *Coniothyrium minitans* on sclerotia of *Sclerotinia sclerotiorum. Can. J. Bot*. 65: 2483–2489.

Huang, H. C. and Kokko, E. G. 1988. Penetration of hyphae of *Sclerotinia sclerotiorum* by *Coniothyrium minitans* without the formation of appressoria. *J. Phytopathol*. 123: 133–139.

Huang, H. C. and Kokko, E. G. 1993. *Trichothecium roseum,* a mycoparasite of *Sclerotinia sclerotiorum. Can. J. Bot*. 71: 1631–1638.

Huang, H. C. and Kozub, G. C. 1989. A simple method for production of apothecia from sclerotia of *Sclerotinia sclerotiorum. Plant Prot. Bull*. 31: 333–345.

Huang, H. C. and Kozub, G. C. 1991a. Monocropping to sunflower and decline of *Sclerotinia* wilt. *Bot. Bull. Acad. Sinica*. 32: 163–170.

Huang, H. C. and Kozub, G. C. 1991b. Temperature requirements for carpogenic germination of sclerotia of *Sclerotinia sclerotiorum* isolates of different geographic origin. *Bot. Bull. Acad. Sinica* 32: 279–286.

Huang, H. C. and Kozub, G. C. 1993. Influence of inoculum production temperature on carpogenic germination of sclerotia of *Sclerotinia sclerotiorum. Can. J. Microbiol*. 39: 548–550.

Huang, H. C. and Kozub, G. C. 1994. Longevity of normal and abnormal sclerotia of *Sclerotinia sclerotiorum. Plant Dis*. 78: 1164–1166.

Huang, H. C. and Sun, S. K. 1991. Effects of S-H mixture or Perlka T M on carpogenic germination and survival of sclerotia of *Sclerotinia sclerotiorum. Soil Biol. Biochem*. 23: 809–813.

Huang, H. C., Kokko, E. G., Yanke, I. J. and Phillippe, R. C. 1993. Bacterial suppression of basal pod rot and end rot of dry peas caused by *Sclerotinia sclerotiorum. Can. J. Microbiol*. 39: 227–233.

Huang, H. C., Huang, J. W., Sainden, G. and Erickson, R. S. 1997a. Effect of allyl alcohol and fermented agricultural wastes on carpogenic germination of sclerotia of *Sclerotinia sclerotiorum* and colonization by *Trichoderma* spp. *Can. J. Plant Pathol*. 19: 43–46.

Huang, H. C., Kokko, E. G. and Erickson, R. S. 1997b. Infection of alfalfa pollen by *Sclerotinia sclerotiorum. Phytoparasitica* 25: 17–24.

Huang, H. C., Chang, C. and Kozub, G. C. 1998. Effect of temperature during sclerotial formation, sclerotial dryness and relative humidity on myceliogenic germination of sclerotia of *Sclerotinia sclerotiorum. Can. J. Bot*. 76: 494–499.

Huang, H. C., Bremer, E., Hynes, R. K. and Erickson, R. S. 2000. Foliar application of fungal biocontrol agents for the control of white mold of dry bean caused by *Sclerotinia sclerotiorum. Biol. Control* 18: 270–276.

Huang, H. C., Erickson, R. S., Chang, C., Moyer, J. R., Larney, F. J. and Huang, J. W. 2002. Organic soil amendments for the control of apothecial production of *Sclerotinia sclerotiorum. Plant Pathol. Bull*. 11: 207–214.

Huang, H. C., Mundel, H. H. and Erickson, R. S. 2003. Effect of physiological resistance and plant architecture on yield of dry bean under disease pressure of white mold (*Sclerotinia sclerotiorum*). *Plant Prot. Bull. Taipei*. 45: 169–176.

Huang, H. C., Erickson, R. S., Chang, C., Moyer, J. R., Larney, F. J. and Huang, J. W. 2005a. Control of white mold of bean caused by *Sclerotinia sclerotiorum* using organic soil amendments and biocontrol agents. *Plant Pathol. Bull*. 14: 183–190.

Huang, H. C., Erickson, R. S., Hezewijk, B. van and Clerck, F. R. de. 2005b. White mold of houndstongue (*Cynoglossum officinale*) caused by *Sclerotinia sclerotiorum* in Canada. *Plant Dis*. 89: 1013.

Huang, H. C., Erickson, R. S., Phillippe, L. M., Mueller, C. A., Sun, S. K. and Huang, J. W. 2006. Control of apothecia of *Sclerotinia sclerotiorum* by soil amendment with S-H mixture or Perlka Red in bean, canola and wheat fields. *Soil Biol. Biochem*. 38: 1348–1352.

Huang, R. C. and Janzen, H. H. 1991. Control of carpogenic germination of sclerotia of *Sclerotinia sclerotiorum* by volatile substance from urea. *Plant Prot. Bull*. [Taiwan] 33: 283–289.

Huang, R. C., Kokko, M. J. and Phillippe, L. M. 1988. White mold of dry bean (*Phaseolus vulgaris* L.) in Southern Alberta. 1983–87. *Can. Plant Dis. Surv*. 68: 11–13.

Huang, X. T. 1992. Control of *Sclerotinia* rot in sunflower. *Plant Prot*. 18: 53.

Huang, Yong Ju, Chen, J. and Li, Y. C. 2000. Genetic study of *Sclerotinia sclerotiorum* resistance in rapeseed (*Brassica napus* L.) I. Its inheritance and combining ability. *Chin. J. Oil Crop Sci*. 22: 1–5.

Hubbard, J. P., Harman, G. E. and Eckenrode, C.J. 1982. Interaction of a biological control agent, *Chaetomium globosum* with seed coat microflora. *Can. J. Microbiol* 28: 431–437.

Hudson, H. J. 1971. The development of the saprophytic fungal flora as leaves senescence and fall. In: *Ecology of Leaf Surface Microorganisms* (T. F. Preece and C. H. Dickinson, eds.). Academic, London, pp. 447–461.

Hudyncia, J., Shew, H. D., Cody, B. R. and Cubeta, M. A. 2000. Evaluation of wounds as a factor to infection of cabbage by ascospores of *Sclerotinia sclerotiorum. Plant Dis*. 84: 316–320.

Humpherson-Jones, F. M. and Cooke, R. C. 1977a. Morphogenesis in sclerotium forming fungi I. Effects of light on *Sclerotinia sclerotiorum, Sclerotium delphinis* and *S. rolfsii. New Phytol*. 78: 171–180.

Humpherson-Jones, F. M. and Cooke, R. C. 1977b. Morphogenesis in sclerotium forming fungi II. Rhythmic production of sclerotia by *Sclerotinia sclerotiorum* (Lib.) de Bary. *New Phytol*. 78: 181–187.

Humpherson-Jones, F. M. and Cooke, R. C. 1977c. Induction of sclerotium formation by acid stalling compounds in *Sclerotinia sclerotiorum* and *Sclerotium rolfsii. Trans. Br. Mycol. Soc*. 68: 413–420.

Hungerford, C. W. and Pitts, R. 1953. The *Sclerotinia* disease of beans in Idaho. *Phytopathology* 43: 519–521.

Hunter, J. E., Abawi, G. S. and Crosier, D. C. 1978. Effects of timing, coverage and spray oil on control of white mold of snap bean with benomyl. *Plant Dis. Reptr*. 62: 633–637.

Hunter, J. E., Dickson, M. H. and Cigna, J. A. 1981. Limited term inoculation; a method to serun bean plants for partial resistance to white mold. *Plant Dis*. 65: 414–417.

Hunter, J. E., Dickson, M. H., Boettger, M. A. and Cigna, J. A. 1982a. Evaluation of plant introductions of *Phaseolus* spp. for resistance to white mold. *Plant Dis*. 66: 320–322.

Hunter, J. E., Steadman, J. R. and Cigna, J. A. 1982b. Preservation of ascospores of *Sclerotinia sclerotiorum* on membrane filters. *Phytopathology* 72: 650–652.

Hunter, J. E., Pearson, R. C., Seem, R. C., Smith, C. A. and Palumbo, D. R. 1984. Relationship between soil moisture and occurrence of *Sclerotinia sclerotiorum* and white mold disease on snap beans. *Prot. Ecol*. 7: 269–280.

Hurrell, G. A., Bourdot, G. W. and Saville, D. J. 2001. Effect of application time on the efficacy of *Sclerotinia sclerotiorum* as a mycoherbicide for *Cirsium arvense* control in pasture. *Biocont. Sci. Technol*. 11: 317–330.

Imolehin, E. D. and Grogan, R. G. 1980. Factors affecting survival of sclerotia and effect of inoculum density, relative position and distance of sclerotia from the host on infection of lettuce by *Sclerotinia minor. Phytopathology* 70: 1162–1167.

Inbar, J., Menendez, A. and Chet, I. 1996. Hyphal interaction between *Trichoderma harzianum* and *Sclerotinia sclerotiorum* and its role in biological control. *Soil Biol. Biochem*. 28: 757–763.

Inglis, G. D. and Boland, G. J. 1990. The microflora of bean and rapeseed petals and the influence of the microflora of bean petals on white mould. *Can. J. Plant Pathol*. 12: 129–134.

Inglis, G. D. and Boland, G. J. 1992. Evaluation of filamentous fungi isolated from petals of bean and rapeseed for suppression of white mould. *Can. J. Microbiol*. 38: 124–129.

Ingold, C. T. 1960. *Dispersal in Fungi*. Clarendon, Oxford, 206 p.

Iqbal, S. M., Mirza, M. S., Bakhsh, A. and Mahmood, T. 1998. *Sclerotinia* rot of peas (*Pisum sativum* L.) in Pakistan. *Sarhad J. Agric*. 14: 157–159.

Iqbal, S. M., Ghafoor, A., Ahmad, Z. and Haqqani, A. M. 2003. Pathogenicity and fungicidal efficacy for *Sclerotinia* rot of brinjal. *Intl. J. Agric. Biol*. 5: 618–620.

Israel, M. S. and Boland, G. J. 1992. Influence of formulation on efficacy of honey bees to transmit biological controls for management of *Sclerotinia* stem rot of canola. *Can. J. Plant Pathol*. 14: 244 (Abstr.).

Ivancia, V. 1992. The influence of the depth of the soil application of sclerotia of *Sclerotinia sclerotiorum* (Lib.) de Bary on its germination capacity. *Cercetari Agronomice in Moldova* 25: 205–208.

Ivancia, V. 1993. A nona planta gazda pentric cruperca *Sclerotinia sclerotiorum* (Lib.) de Bary, in Romania. *Probleme de Protectia Plantelar* 21: 35–36.

Ivancia, V., Voicu, M. and Raranciuc, S. 1998. Researches on reduction of *Sclerotinia sclerotiorum* attack in sunflower by *Stachybotris lobulata* (Berk.). *Probleme de Protectia Plantelor* 26: 39–44.

Iwata, I. and Igita, K. 1972. On the growth characteristics of direct sowing rape on upland field. *Bull. Kyushu Agric. Exp. Stn*. 16: 207.

Jackson, I. F. and Smith, B. N. 1979. Procymidone evaluation for the control of *Sclerotinia* spp. and *Botrytis* spp. In: *Proceedings of the 32nd New Zealand Weed and Pest Control Conference*, pp. 278–283.

Jagger, I. C. 1913. The small lettuce *Sclerotinia* an undescribed species. *Phytopathology* 3: 74. (Abstr.).

Jagger, I. C. 1920. *Sclerotinia minor* n. sp., the cause of a decay of lettuce, celery and other crops. *J. Agric. Res*. 20: 331–334.

Jamalainen, E. A. 1954. Over-wintering of cultivated plants under snow. *F.A.O. Plant Prot. Bull*. 2: 12.

Jamaux, J. and Spire, D. 1994. Development of a polyclonal-antibody based immunoassay for the early detection of *Sclerotinia sclerotiorum* in rapeseed seeds. *Plant Pathol*. 43: 847–862.

Jamaux, I., Gelic, B. and Lamarque, C. 1995. Early stages of infection of rapeseed petals and leaves by *Sclerotinia sclerotiorum* revealed by SEM. *Plant Pathol*. 44: 22–30.

Jancys, Z. 1998. Relationship between the resistance of red clover, *Trifolium pratense* L., to oxalate and to *Sclerotinia trifoliorum* Erikss. Biotechnologija augalu selekcijoje. Moksline konferencija, Lietuvos zemes ukio universitetas, 1998 m. spalio 8–9 d. pp. 71–76.

Jarvis, W. R. and Hawthorne, B. T. 1972. *Sclerotinia minor* on lettuce: progress of an epidemic. *Ann. Appl. Biol*. 70: 207–214.

Jedryczka, M., Lewartowska, E., Frencel, I. and Drobnik, M. 1996. Evaluation of resistance of polish oilseed winter rape cultivars to stem canker and *Sclerotinia* stem rot. *Plant Breed. Seed Sci*. 40: 17–23.

Jiang, Dao Hong, Li, G. Q., Wang, D. B. and Zhou, Q. 2000. The sexual inheritance of cultural characteristics of *Sclerotinia sclerotiorum* hypovirulent isolate Ep-1PN. *Mycosystema* 19: 236–240.

Jiao, H. S., Cheng, Z. M., Xu, X. H., Zou, L. B. Lu, X. D., Ceng, P. F., Liu, J. P. and Ding, T. F. 1994. Studies on evaluation for resistance of soybean germplasm resource to *Sclerotinia sclerotiorum* (Lib.) de Bary. *Soybean Sci*. 13: 349–356.

Jnr, M. L., Lopes, C. A. and Silva, W. L. C. 2000. *Sclerotinia* rot losses in processing tomatoes grown under centre pivot irrigation in Central Brazil. *Plant Pathol*. 49: 51–56.

Jones, D. 1970. Ultrastructure and composition of the cell wall of *Sclerotinia sclerotiorum. Trans. Br. Mycol. Soc*. 54: 351–360.

Jones, D. 1973. Factors affecting germination of sclerotia of *Sclerotinia sclerotiorum* from peas. *Trans. Br. Mycol. Soc*. 60: 495–500.

Jones, D. 1974a. Ultrastructure of the stipe and apothecium of *Sclerotinia sclerotiorum. Trans. Br. Mycol. Soc*. 63: 386–389.

Jones, D. 1974b. Fungicidal effects of the fumigant Dazomet on sclerotia of *Sclerotinia sclerotiorum* in soil. *Trans. Br. Mycol. Soc*. 63: 249–254.

Jones, D. 1976. Infection of plant tissue by *Sclerotinia sclerotiorum:* a scanning electron microscope study. *Micron*. 7: 275–279.

Jones, D. and Gray, E. G. 1973. Factors affecting germination of sclerotia of *Sclerotinia sclerotiorum* from peas. *Trans. Br. Mycol. Soc*. 60: 495–500.

Jones, D. and Gray, E. G. 1977. Crop losses due to the soil borne fungus, *Sclerotinia sclerotiorum. ARC Res. Rev*. 3: 79–80.

Jones, D. and Watson, D. 1969. Parasitism and lysis by soil fungi of *Sclerotinia sclerotiorum* (Lib.) de Bary, a phytopathogenic fungus. *Nature* (Land.) 224: 287–288.

Jones, D., Gordon, A. H. and Bacon, J. S. D. 1974. Cooperative action by endo and exo-β (1–3)-glucanases from parasitic fungi in the degradation of cell wall glucans of *Sclerotinia sclerotiorum. Biochem. J*. 140: 47–55.

Jones, E., Whipps, J. M., Elad, Y. (ed.), Freeman, S. (ed.) and Monte, E. 2001. Biological control of *Sclerotinia sclerotiorum* in glasshouse lettuce. IOBC-WPRS Working Group "*Biological Control of Fungal and Bacterial Plant Pathogens*". In: *Proceedings of Sixth meeting, Biocontrol Agents: Mode of Action and Interaction with Other Means of Control*, Sevilla, Spain, November 30–December 3, 2000. *Bull. OILB-SROP* 24: 83–87.

Jones, E. E. and Whipps, J. M. 2002. Effect of inoculum rates and sources of *Coniothyrium minitans* on control of *Sclerotinia sclerotiorum* disease in glasshouse lettuce. *Eur. J. Plant Pathol*. 108: 527–538.

Jones, E. S. 1923. Taxonomy of the *Sclerotinia* on *Helianthus annuus* L. *Phytopathology* 13: 496.

Jons, V. L., Venette, J. R., Lamey, H. A. and Mc Mullen, M. P. 1996. First report of *Sclerotinia sclerotiorum* on Salvia in the United States. *Plant Dis*. 80: 1302.

Jurick, W. M. I. I., Dickman, M. B. and Rollins, J. A. 2004. Characterization and functional analysis of a cAMP dependent protein kinase A catalytic subunit gene (pka 1) in *Sclerotinia sclerotiorum. Physiol. Mol. Plant. Pathol*. 64: 155–163.

Jurkovic, D. and Culek, M. 1997. *Abutilon theophrasti* Medik- a new host for *Sclerotinia sclerotiorum* (Lib.) de Bary in Croatia. *Acta Phytopathologica et Entomologica Hungarica*. 32: 307–312.

Kakoti, R. K. and Saikia, U. N. 1997. Sclerotial survival of *Sclerotinia sclerotiorum* (Lib.) de Bary in soil. *J. Agric. Sci. Soc. North-East India* 10: 265–267.

Kamara, A. M., Aboshosha, S. S. and Saeed, F. A. 1991. Effects of pathogenesis of *Sclerotinia sclerotiorum* on soluble protein in infected sunflower tissues. *Asscut. J. Agric. Sci*. 22: 65–80.

Kamensky, M., Ovadis, M., Chet, I. and Chernin, L. 2003. Soil-borne strain IC14 of *Serratia plymuthica* with multiple mechanisms of antifungal activity provides biocontrol of *Botrytis cinerea* and *Sclerotinia sclerotiorum* diseases. *Soil Biol. Biochem*. 35: 323–331.

Kanbe, M., Koga, H., Fujimoto, F., Okumura, K. and Mizuno, K. 1997a. Growth conditions of alfalfa for screening of resistance to *Sclerotinia trifoliorum* and microscopic observations of infected tissues. *Res. Bull. Aichi ken Agril. Res. Cent*. 29: 362.

Kanbe, M., Fujimoto, F., Mizukami, Y., Inami, S. and Fukaya, K. 1997b. Increase of resistance of alfalfa to *Sclerotinia* crown and stem rot through recurrent selection. *Breed. Sci*. 47: 347–351, 391.

Kanbe, M., Mizukami, Y. and Fujimoto, F. 2002. Improvement of resistance to *Sclerotinia* crown and stem rot of alfalfa through phenotypic recurrent selection. *JARQ Japan Agric. Res*. (*Quarterly*) 36: 1–5.

Kang, I. S. and Chahal, S. S. 2000. Prevalence and incidence of white rot of rapeseed and mustard incited by *Sclerotinia sclerotiorum* in Punjab. *Plant Dis. Res*. 15: 232–233.

Kanno, H. and Ohkubo, H. 1999. *Sclerotinia* rot of Malabar nightshade (*Basella rubra* L.), *Lapsana apogonoides* Maxim., Bishop's weed (*Ammi majus* L.), blue lace flower (*Didiscus caeruleus* DC.) and *Portulaca oleracea* L. caused by *Sclerotinia sclerotiorum. Ann. Reprt. Soc. Plant Prot. North Japan*. 50: 115–119.

Kapil, R. and Kapoor, A. S. 2005. Management of white rot of pea incited by *Sclerotinia sclerotiorum* using *Trichoderma* spp. and biobesticides. *Indian Phytopath*. 58: 10–16.

Kapoor, A. S., Singh, D. and Singh, B. M. 1990a. A technique for artificial inoculation of pea seedling with *Sclerotinia sclerotiorum. Indian Phytopath*. 43: 469–470.

Kapoor, K. S. 1983. Some aspects of the host parasite relations between *Sclerotinia sclerotiorum* (Lib.) de Bary and rapeseed. *6th Int. Rapeseed Conf.* Paris, May 17–19, p. 188 (Abstr.).

Kapoor, K. S. 1994. Stipe regeneration potential of sclerotia of *Sclerotinia sclerotiorum* (Lib.) de Bary. *Plant Dis. Res*. 9: 98–100.

Kapoor, K. S., Gill, H. S. and Sharma, S. R. 1986. A technique for artificial inoculation of curd stage of cauliflower with *Sclerotinia sclerotiorum. Cruciferae Newslett*. 11: 13.

Kapoor, K. S., Sharma, S. R. and Gangopadhyay, S. 1990b. Assessment of resistance in eggplants against *Sclerotinia* wilt with a new screening technique. *Capsicum Newslett*. 8–9: 70.

Kars, I. and van Kan, J. A. L. 2004. Extracellular enzymes and metabolites involved in pathogenesis of *Botrytis*. In: *Botrytis: Biology, Pathology and Control* (Y. Elad, B. Williamson, P. Tudzynski and N. Delen, eds.). Kluwer, Dordrecht, The Netherlands, pp. 99–118.

Kars, I., Krooshof, G. H., Wagemakers, L., Joosten, R., Benen, J. A. E. and van Kan, J. A. L. 2005. Necrotizing activity of five *Botrytis cinerea* endopolygalacturonases produced in *Pichia pastoris. Plant J*. 43: 213–225.

Kasza, Z., Vagvolgyi, C., Fevre, M. and Cotton, P. 2004. Molecular characterization and *in planta* detection of *Sclerotinia sclerotiorum* endopolygalacturonase genes. *Curr. Microbiol*. 48: 208–213.

Keay, M. A. 1939. A study of certain species of the genus *Sclerotinia. Ann. Appl. Biol*. 26: 227–246.

Keon, J. P. R., Byrde, R. J. W. and Cooper, R. M. 1987. Some aspects of fungal enzymes that degrade plant cell walls. In: *Fungal Infection of Plants* (G. F. Pegg and P. G. Ayres, eds.). Cambridge University Press, Cambridge, pp. 133–157.

Kerr, K. D., Steadman, J. R. and Nelson, L. A. 1978. Estimation of white mold disease reduction of yield and yield components of dry edible beans. *Crop Sci*. 18: 275–279.

Kesarwani, M., Azam, M., Natarajan, K., Mehta, A. and Datta, A. 2000. Oxalate decarboxylase from *Collybia velutipes*. Molecular cloning and its over expression to confer resistance to fungal infection in transgenic tobacco and tomato. *J. Biol. Chem*. 275: 7230–7238.

Khalil, F. A. and Ragab, M. M. 1990. The behavior and survival of *Sclerotinia sclerotiorum* in relation to sclerotial lipid contents. *Egypt. J. Phytopathol*. 20: 107–116.

Khare, K. B. and Bompeix, G. 1976. Activities proteolytiques des *Sclerotinia sclerotiorum* et *S. minor*: role possible loss de la pathogenese. *Rev. Mycol*. 40: 65–84 (Paris).

Kim, H. S. and Diers, B. W. 2000. Inheritance of partial resistance to *Sclerotinia* stem rot in soybean. *Crop Sci*. 40: 55–61.

Kim, H. S., Sneller, C. H. and Diers, B. W. 1999. Evaluation of soybean cultivars for resistance to *Sclerotinia* stem rot in field environments. *Crop Sci*. 39: 64–68.

Kim, H. S., Hartman, G. L., Manandhar, J. B., Graef, G. L., Steadman, J. R. and Diers, B. W. 2000. Reaction of soybean cultivars to *Sclerotinia* stem rot in field, greenhouse and laboratory evaluations. *Crop Sci*. 40: 665–669.

Kim, K. G. 1976. Effect of light on the sclerotial formation of *Sclerotinia sclerotiorum* (Lib.) de Bary. *Kor. J. Plant Prot*. 15: 153–159.

Kirkegaard, J. A., Robertson, M. J., Hamblin, P. and Sprague, S. J. 2006. Effect of blackleg and *Sclerotinia* stem rot on canola yield in the high rainfall zone of southern New South Wales, Australia. *Aust. J. Agril. Res*. 57: 201–212.

Kiryakov, I., Genchev, D. and Stanoeva, Y. 2002. Resistance of domestic and introduced bean accessions (*Phaseolus vulgaris* L.) to white mold (*Sclerotinia sclerotiorum*). *Rasteniev dni Nauki*. 39: 333–337.

Kishore, M. A. and Gupta, J. P. 1997. Organic solvents for enhanced efficacy of carbendazim against *Sclerotinia* rot of sunflower. *Indian Phytopath*. 50: 246–249.

Klasse, H. J. 1993. Successful control of *Sclerotinia* and clubroot. *Gemiise* (Miinchen) 29: 389–390.

Knudsen, G. R., Eschen, D. J., Dandurand, L. M. and Bin, L. 1991. Potential for biocontrol of *Sclerotinia sclerotiorum* through colonization of sclerotia by *Trichoderma harzianum. Plant Dis*. 75: 466–470.

Koch, E. F. A. and Menten, J. O. M. 2000. An alternative method for detection of *Sclerotinia sclerotiorum* in common bean seeds. *Summa Phytopathologica* 26: 276–279.

Kochenkova, K. G. and Polyakov, P. V. 1971. The effect of fertilizers on infection of sunflower by white rot. *Rev. Plant. Pathol*. 50: 2414 (Abstr.).

Kohli, U., Dogra, R. C., Kapoor, K. K. and Sharma, P. K. 2006. Chitinolytic *Pseudomonas maltophila* antagonistic to root rot pathogens of sunflower (*Helianthus annuus*). *Indian J. Microbiol*. 46: 45–49.

Kohli, Y. and Kohn, L. M. 1996. Mitochondrial haplotype in population of the plant infecting fungus *Sclerotinia sclerotiorum*: wide distribution in agriculture, local distribution in the wild. *Mol. Ecol*. 5: 773–783.

Kohli, Y. and Kohn, L. M. 1998. Detection of random association among loci in clonal populations of *Sclerotinia sclerotiorum. Fungal Genet. Biol*. 23: 139–149.

Kohli, Y., Morrall, R. A. A., Anderson, J. B. and Kohn, L. M. 1992. Local and trans-Canadian clonal distribution of *Sclerotinia sclerotiorum* on Canola. *Phytopathology* 82: 875–880.

Kohli, Y., Brunner, L. J., Yoell, H., Milgroom, M. G., Anderson, J. B., Morrall, R. A. A. and Kohn, L. M. 1995. Clonal dispersal and spatial mixing in population of plants and pathogenic fungus *Sclerotinia sclerotiorum. Mol. Ecol*. 4: 69–77.

Kohn, L. M. 1979a. A monographic revision of the genus *Sclerotinia. Mycotaxon* 9: 365–444.

Kohn, L. M. 1979b. Delimitation of the economically important plant pathogenic *Sclerotinia* species. *Phytopathology* 69: 881–886.

Kohn, L. M. 1992. Developing new characters for fungal systematic: An experimental approach for determining the rank of *Sclerotinia. Mycologia* 84: 139–153.

Kohn, L. M. 2001. Integrating our genotypic diversity data: towards a global picture of population subdivision and fine scale structure in *Sclerotinia* with implications for disease management. In: *Proceedings of Sclerotinia 2001, the XI International Sclerotinia Workshop* (C. S. Young and K. J. D. Hughes, eds.). York 8–12, July 2001, Central Science Laboratory, York, England, pp. 17–19.

Kohn, L. M. and Korf, R. P. 1975. Variation in ascomycetes iodine reactions: KOH pretreatment explored. *Mycotaxon* 3: 165–172.

Kohn, L. M., Carbone, I. and Anderson, J. B. 1990. Mycelial interactions in *Sclerotinia sclerotiorum. Expl. Mycol*. 14: 255–267.

Kohn, L. M., Petsche, D. M., Bailey, S. R., Novak, L. A. and Anderson, J. B. 1988. Restriction fragment length polymorphisms in nuclear and mitochondrial DNA of *Sclerotinia* species. *Phytopathology* 78: 1047–1951.

Kohn, L. M., Stasovski, E., Carbone, I., Royer, J. and Anderson, J. B. 1991. Mycelial incompatibility and molecular markers in identify genetic variability in field population of *Sclerotinia sclerotiorum. Phytopathology* 81: 480–485.

Koike, S. T. 1997. Occurrence of stem and crown rot of *Gaillardia grandiflora* caused by *Sclerotinia sclerotiorum* in California. *Plant Dis*. 81: 1334.

Koike, S. T. 1999. Stem and crown rot of chervil caused by *Sclerotinia sclerotiorum* in California. *Plant Dis*. 83: 1177.

Koike, S. T. 2003. *Sclerotinia* stem and crown rot of corm salad caused by *Sclerotinia minor* in California. *Plant Dis*. 87: 1264.

Koike, S. T. and O' Brien, R. D. 1995. Basil as a host of *Sclerotinia minor. Plant Dis*. 79: 859.

Koike, S. T. and Subbarao, K. V. 1995. First report of radicchio as a host of *Sclerotinia minor. Plant Dis*. 79: 966.

Koike, S. T., Gonzales, T. G., Vidauri, M. and Subbarao, K. V. 1994. First report of *Sclerotinia minor* as a pathogen of cauliflower in California. *Plant Dis*. 78: 1216.

Koike, S. T., Smith, R. F., Jackson, L. E., Wyland, L. J., Inman, J. I. and Chaney, W. E. 1996. Phacelia, Lana woollypod vetch and Austrian winter pea: three new cover crop hosts of *Sclerotinia minor* in California. *Plant Dis*. 80: 1409–1412.

Koike, S. T., Daugovish, O. and Downer, J. A. 2006. *Sclerotinia* petiole and crown rot of celery caused by *Sclerotinia minor* in California. *Plant Dis*. 90: 829.

Kolattukudy, P. E. 1985. Enzymatic penetration of the plant cuticle by fungal pathogens. *Ann. Rev. Phytopath*. 23: 223–250.

Kolkman, J. M. and Kelly, J. D. 2000. An indirect test using oxalate to determine physiological resistance to white mold in common bean. *Crop Sci*. 40: 281–285.

Kolkman, J. M. and Kelly, J. D. 2003. QTL conferring resistance and avoidance to white mold in common bean. *Crop Sci*. 43: 539–548.

Kolte, S. J. 1985a. *Diseases of Annual Edible Oilseed Crops: Sunflower and Groundnut Diseases*. Vol. I, CRC, Boca Raton, FL, Pp. 135.

Kolte, S. J. 1985b. *Diseases of Annual Edible Oilseed Crops: Rapeseed-Mustard and Sesamum Diseases*. Vol. II, CRC, Boca Raton, FL, pp. 135.

Kolte, S. J. 1985c. *Diseases of Annual Edible Oilseeds Crops: Sunflower*. Vol. III, CRC, Boca Raton, FL, p. 51.

Kolte, S. J. and Mukhopadhyay, A. N. 1973. Occurrence of some new sunflower diseases in India. *PANS* 19: 392.

Kolte, S. J. and Tewari, A. N. 1977. Note on effect of planting date on occurrence and severity of sunflower diseases. *Pantnagar J. Res*. 2: 236.

Kolte, S. J., Singh, B. D. and Tewari, A. N. 1976. Evaluation of sunflower genotypes for resistance to *Sclerotinia sclerotiorum. Indian J. Mycol. Plant Pathol*. 6: 65–67.

Koponen, H. and Valkonen, J. P. T. 1996. Unique symptoms on stored carrots and white mold on hairy vetch caused by *Sclerotinia sclerotiorum* in Finland. *Plant Dis*. 80: 599.

Kora, C., Boland, G. J. and McDonald, M. R. 2002. First report of foliar and root infection of carrot by *Sclerotinia minor* in Ontario, Canada. *Plant Dis*. 86: 1406.

Kora, C., McDonald, M. R. and Boland, G. J. 2003. *Sclerotinia* rot of carrot: An example of phonological adaptation and bicyclic development by *Sclerotinia sclerotiorum. Plant Dis*. 87: 456–470.

Kora, C., McDonald, M. R. and Boland, G. J. 2005a. Lateral clipping of canopy influences the microclimate and development of apothecia of *Sclerotinia sclerotiorum* in carrots. *Plant Dis*. 89: 549–557.

Kora, C., McDonald, M. R. and Boland, G. J. 2005b. Epidemiology of *Sclerotinia* rot of carrot caused by *Sclerotinia sclerotiorum. Can. J. Plant Pathol*. 27: 245–258.

Korf, R. P. 1973. Discomycetes and tuberales. In: *The Fungi - An Advanced Treatise* (G. G. Ainsworth, F. K. Sparrow and A. S. Sussman, eds.). Vol. 4A, Academic, New York, pp. 249–319.

Korf, R. P. and Dumont, K. P. 1968. The case of *Lambertella brumeola*: an object lesson in taxonomy of the higher fungi. *J. Elishn Mitchell Sci. Soc*. 84: 242–247.

Korf, R. P. and Dumont, K. P. 1972. *Whetzelinia*, a new generic name for *Sclerotinia sclerotiorum* and *Sclerotinia tuberosa. Mycologia* 64: 248–251.

Korf, R. P. and Willetts, H. J. 1975. Ontogenetic and histochemical studies of the apothecium of *Sclerotinia sclerotiorum. Ann. Bot*. 39: 185–191.

Kosasih, B.D. and Willetts, H.J. 1975. Types of abnormal apothecia produced by *Sclerotinia sclerotiorum*. *Mycologia* 67: 89–97.

Kreitlow, K. W. 1949. *Sclerotinia trifoliorum*, a pathogen of ladino clover. *Phytopathology* 39: 158–166.

Krietlow, K. W. 1953. Infection studies with dried grain inoculum of *Sclerotinia trifoliorum. Phytopathology* 41: 553–558.

Kreitlow, K. W. and Sprague, V. G. 1951. Effect of temperature on growth and pathogenicity of *Sclerotinia sclerotiorum. Phytopathology* 41: 752–757.

Krexner, R. 1969. Sclerotial disease of sunflower. *Rev. Appl. Mycol*. 48: 664 (Abstr.).

Krikun, J., Papvizas, G. C. and Frank, Z. 1980. Application of metham through sprinkler irrigation for control of soil-borne pathogens of peanuts. *Proc. Am. Peanut Res. Educ. Soc*. 12: 42.

Kritzman, G., Chet, I. and Henis, Y. 1977. The role of oxalic acid in the pathogenic behaviour of *Sclerotinia rolfsii* Sacc. *Exp. Mycol*. 1: 280–285.

Kruger, W. 1973. Control measures for *Sclerotinia sclerotiorum* in rape. *Phytopath. Z.* 77: 125–137.

Kruger, W. 1974. Investigation concerning the epidemiology of rape cankers giving rise to *Sclerotinia sclerotiorum* (Lib.) de Bary. In: *Proceedings of the International Rapeseed Conference, 4 Giessen*. West Germany, pp. 595–603.

Kruger, W. 1975a. Influence of more environmental factors on the development of apothecia and ascospores of *Sclerotinia sclerotiorum* (Lib.) de Bary. *Z. Pflimzenkaran Kh Planzensehtz*. 82: 101–108.

Kruger, W. 1975b. Influence of weather on the attack of rape by *Sclerotinia sclerotiorum* Lib.) de Bary. *Nachrichtenble Devet. Pflanzenschutzd*. (*Braunschweig*) 27: 1–6.

Kruger, W. 1976. The influence of environmental factors on the apothecial development of *Sclerotinia sclerotiorum* (Lib.) de Bary. *Nachrichtenblatt Deut. Pflanzensehtzd*. (*Braunschweig*.) 28: 130–135.

Kruger, W. 1980. On the effect of calcium cyanamide on the development of apothecia of *Whetzelinia sclerotiorum* (Lib.) Korf and Dumont, the agent of stalk rot of rape. *Rev. Plant. Pathol*. 59: 5438 (Abstr.).

Kruger, W. and Stoltenberg, J. 1983. Control of rape diseases II. Measures for disease reduction caused by *Sclerotinia sclerotiorum* with consideration to economical aspects. *Phytopath. Z.* 108: 114–126.

Kruger, W., Marguard, W. R. and Schosser, E. 1981. Plant disease product quality. II. Influence of stem canker *Sclerotinia sclerotiorum* (Lib.) de Bary on the quality of rapeseed. *Rev. Plant Pathol*. 60: 2949 (Abstr.).

Kuleshova, M. K. 1990. Presowing treatment of pea seeds. *Zashchita Rastenii* (Moskva) 12: 39.

Kulik, M. M. 1995. The potential for using *Cyanobacteria* (blue-green algae) and algae in the biological control of plant pathogenic bacteria and fungi. *Eur. J. Plant Pathol*. 101: 585–599.

Kull, L. S., Vuong, T. D., Powers, K. S., Eskridge, K. M., Steadman, J. R. and Hartman, G. L. 2003. Evaluation of resistance screening methods for *Sclerotinia* stem rot of soybean and dry bean. *Plant Dis*. 87: 1471–1476.

Kull, L. S., Pederson, W. L., Palmquist, D. and Hartman, G. L. 2004. Mycelial compatibility groupings and aggressiveness of *Sclerotinia sclerotiorum. Plant Dis*. 88: 325–332.

Kumar, B., Chahal, S. S. and Ahuja, K. L. 1998. Effect of *Sclerotinia* head rot on some bio-constituents of sunflower seed. *Indian Phytopath*. 51: 359–360.

Kurian, P. and Stelzig, D. A. 1979. The synergistic role of oxalic acid and endopolygalacturonase in bean leaves infected by *Cristulariella pyramidalis. Phytopathology* 69: 1301–1304.

Kushal, R. and Saharan, G. S. 1994. Seed mycoflora of sunflower and its effect on seed and plant health. *Indian J. Mycol. Plant Pathol*. 24: 15–19.

Kwon, J. H. and Park, C. S. 2002. Occurrence of *Sclerotinia* rot of *Leonurus sibiricus* caused by *Sclerotinia sclerotiorum. Res. Plant Dis*. 8: 113–116.

Laclerca, P. 1973. Influence de facteurs hereditairs sur la resistance apparente du edible beans. *Crop Sci*. 18: 275–279.

Lamarque, C. 1983. Climatic conditions for infection of sunflower by *Sclerotinia sclerotiorum,* forecasting of local epidemic. *Bull. DEPP* 13: 75–78.

Lamey, H. A., Nelson, B. D. (ed.) and Gulya, T. J. 1998. Incidence of *Sclerotinia* stem rot on canola in North Dakota and Minnesota, 1991–1997. In: *Proceedings of 1998 International Sclerotinia Workshop*, Fargo, ND, 9–12 September, pp. 7–9.

Lane, B. G., Dunwell, J. M., Ray, J. A., Schmitt, M. R. and Cuming, A. C. 1993. Germin, a protein marker of early plant development is an oxalate oxidase. *J. Biol. Chem*. 268: 12239–12242.

Langston, D. B. Jr., Phipps, P. M. and Stipes, R. J. 2002. An algorithm for predicting outbreaks of *Sclerotinia* blight of peanut and improving the timing of fungicide sprays. *Plant Dis*. 86: 118–126.

Latorre, B. A. and Guerrero, M. J. 2001. First report of shoot blight of grapevine caused by *Sclerotinia sclerotiorum* in Chile. *Plant Dis*. 85: 1122.

Lauritzen, J. I. 1932. Development of certain storage and transit diseases of carrot. *J. Agric Res*. 44: 861–912.

Lazar, A., Hatman, M., Georgescu, T., Lacob, V. and Lesan, V. 1989. Methods of preventing pathogen attacks in sunflowers BPP. *Buletinul de Protectia Plantelor*. 2: 35–44.

Lazzaretti, E., Menten, J. O. M. and Bettiol, W. 1994. *Bacillus subtilis* antagonistic to the principal pathogens associated with bean and wheat seeds. *Fitopathologia Venezolana* 7: 42–46.

Lee, C. D., Renner, K. A., Penner, D., Hammerschmidt, R. and Kelly, J. D. 2005. Glyphosate resistant soybean management system effect on *Sclerotinia* stem rot. *Weed Technol*. 19: 580–588.

Lee, Y. A. and Wu, W. S. 1979. Management of the *Sclerotinia* disease with biological and chemical methods. *Memorial Coll. Agric. Nat. Taiwan Univ., Taipei* [Taiwan] 19: 96–108.

Lefol, C. and Morrall, R. A. A. 1996. Immunofluorescent staining of *Sclerotinia* ascospores on canola petals. *Can. J. Plant Pathol*. 18: 237–241.

Lemay, A. V., Bailey, J. E. and Shew, B. B. 2002. Resistance of peanut to *Sclerotinia* blight and the effect of acibenzolar-S-methyl and fluazinam on disease incidence. *Plant Dis*. 86: 1315–1317.

Lemke, P. A. and Nash, C. H. 1974. Fungal viruses. *Bacterial Rev*. 38: 29–56.

Leon, J., Lawton, M. A. and Raskin, I. 1995. Hydrogen peroxide stimulates salicylic acid biosynthesis in tobacco. *Plant Physiol*. 108: 1673–1678.

Leone, G. and Tonneijck, A. E. G. 1990. A rapid procedure for screening the resistance of bean cultivars (*Phaseolus valgaris* L) to *Botrysis cinerea* and *Sclerotinia sclerotiorum. Euphytica* 48: 87–90.

Lester, E. and Large, E. C. 1958. Surveys of clover rot with incidental observations on eelworm in clover, England and Wales. 1953–1955. *Plant Pathol*. 7: 115–124.

Letham, D. B. 1975. Stimulation by light of apothecial initial development of *Sclerotinia sclerotiorum. Trans Br. Mycol. Soc*. 65: 333–335.

Letham, D. B., Huett, D. D. and Trimboli, D. S. 1976. Biology and control of *Sclerotinia sclerotiorum* in cauliflower and tomato crops in coastal New South Wales. *Plant Dis. Reptr*. 60: 286–289.

LeTourneau, D. 1966. Trehalose and acyclic polyols in sclerotia of *Sclerotinia sclerotiorum. Mycologia* 58: 934–942.

LeTourneau, D. 1976. Effect of phenylthiourea on growth and sclerotial formation of *Sclerotium rolfsii* and *Whetzelinia sclerotiorum. Can. J. Microbiol*. 22: 379–383.

LeTourneau, D. 1979. Morphology, cytology and physiology of *Sc1erotinia* species in culture. *Phytopathology* 69: 887–890.

Levine, A., Tenhaken, R., Dixon, R. and Lamb, C. 1994. H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. *Cell* 79: 583–593.

Lewis, B. G. and Garrod, B. 1983. Carrots. In: *Post Harvest Pathology of Fruits and Vegetables* (C. Dennis, ed.). Academic Press, London, pp. 103–124.

Lewthwaite, S. L. and Wright, P. J. 2005. Sweet potato vine resistance to *Sclerotinia* rot. *Acta Hort*. 670: 63–69.

Li, G. Q., Wang, D. B., Jiang, D. H., Huang, H. Z. and Zhou, Q. 1998. A new kind of lettuce drop caused by *Sclerotinia* sp. in central China. *Acta Phytopathol. Sinica* 28: 249–255.

Li, G. Q., Wang, D. B., Jiang, D. H., Huang, H. C. and Laroche, A. 2000. First report of *Sclerotinia Navalis on lettuce in central China. Mycol. Res*. 104: 232–237.

Li, G. Q., Huang, H. C. and Acharya, S. N. 2003a. Antagonism and biocontrol potential of *Urocladium atrum* on *Sclerotinia sclerotiorum. Biol. Control* 28: 11–18.

Li, G. Q., Huang, H. C., Laroche, A. and Acharya, S. N. 2003b. Occurrence and characterization of hypovirulence in the tan sclerotial isolate S10 of *Sclerotinia sclerotiorum. Mycol. Res*. 107: 1350–1360.

Li, H. and Leifert, C. 1994. Development of resistance in *Botryotinia fuckeliana* (de Bary) Whetzel against the biological control agent *Bacillus subtilis* CL27. *Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz*. 101: 414–418.

Li, Hong Xia and Zhou, M. G. 2004. Rapid identification of carbendazim resistant strains of *Sclerotinia sclerotiorum* using allele-specific oligonucleotides (ASO)-PCR. *Scientia Agricultura Sinica* 37: 1396–1399.

Li, Hong Xia, Zhou, M. G. and Lu, Y. J. 2002. Using polymerase chain reaction for detection of carbendazim resistance in *Sclerotinia sclerotiorum. Mycosystema* 21: 370–374.

Li, Hong Xia, Lu, Y. J., Zhou, M. G. and Wang, X. F. 2003. Mutation in beta-tubulin of *Sclerotinia sclerotiorum* as related to carbendazim resistance in the rapeseed field isolates of the pathogen. *Chin. J. Oil Crop Sci*. 25: 56–60.

Li, MoXiao, Gong, X. Y., Zheng, J., Jiang, D. H., Fu, Y. P. and Hou, M. S. 2005. Transformation of *Coniothyrium minitans*, a parasite of *Sclerotinia sclerotiorum* with *Agrobacterium tumefaciens. FEMS Microbiol. Lett*. 243: 323–329.

Li, R., Rimmer, R., Buchwaldt, L., Sharpe, A. G., Senguin-Swartz, G. and Hegedus, D. D. 2004a. Interaction of *Sclerotinia sclerotiorum* with *Brassica napus* cloning and characterization of endo and exo-polygalacturonases expressed during saprophytic and parasitic modes. *Fungal Genet. Biol*. 41: 754–765.

Li, Ru Gang, Rimmer, R., Buchwaldt, L., Sharpe, A. G., Seguin Swartz, G., Coutu, C. and Hegedus, D. D. 2004b. Interaction of *Sclerotinia sclerotiorum* with a resistant *Brassica napus* cultivar: expressed sequence tag analysis identifies genes associated with fungal pathogenesis. *Fungal Genet. Biol*. 41: 735–753.

Li, Shi Dong, Miao, Zuo Qing, Zhang, Yong Hua and Liu, Xing Zhong. 2003. *Monacrosporium janus* sp. nov., a new nematode trapping hyphomycetes parasitizing sclerotia and hyphae of *Sclerotinia sclerotiorum. Mycol. Res*. 107: 888–894.

Li, Y. B., Yongli, Z. and Nian, L. B. 1994. Study on the dissemination distance of sunflower stem rot fungus. *Plant Prot*. 20: 12–13.

Li, Y. H., Wang, H., Li, J. C., Wang, D. and Li, D. R. 2005. Infection of *Sclerotinia sclerotiorum* to rapeseed, soybean and sunflower and its virulence differentiation. *Acta Phytopathol. Sinica* 35: 486–492.

Libert, M.A. 1837. Plante crytogamicae arduennae (Exsiccate) no. 326. Published by the author.

Liew, C. L. and Prange, R. K. 1994. Effect of ozone and storage temperature on post harvest diseases and physiology of carrots (*Daucus carota* L). *J. Am. Soc. Hort. Sci*. 119: 563–567.

Lithourgidis, A. S., Travella-Kloneri, K. and Roupakias, D. G. 1989. Methods of inoculation of faba bean plants in *Sclerotinia sclerotiorum. J. Phytopathol*. 127: 123–128.

Lithourgidis, A. S., Roupakias, D. G. and Damalas, C. A. 2005. Inheritance of resistance to *Sclerotinia* stem rot (*Sclerotinia trifoliorum*) in faba beans (*Vicia faba* L.). *Field Crops Res*. 91: 125–130.

Lithourgidis, A. S., Roupakias, D. G. and Travella-Kloneri, K. 2007. Stem rot disease incidence on faba beans in an artificially infested field. *J. Plant Dis. Prot*. 114: 120–125.

Litkei, J. 1988. Occurrence of *Sporidesmium sclerotivorum* Uecker, Ayers and Adams in Hungary. *Acta Phytopathol. Entomol. Hung*. 23: 115.

Liu, C. G., Du, D. Z., Zou, C. S. and Huang, Y. J. 1990. Initial studies on tolerance to *Sclerotinia sclerotiorum* (Lib.) de Bary in *Brassica napus* L. *Proceedings of Symposium of China International Rapeseed Science*, 70–71.

Liu, H. Y. 1989. Hyperparasitism of *Gliocladium deliquescens* and *Penicillium vermiculatum* on *Sclerotinia sclerotiorum. J. Plant Prot. Bull*. [Taiwan] 31: 175–184.

Liu, H. Y. and Sun, S. K. 1984. Some ecological studies of *Sclerotinia sclerotiorum*, the casual agent *Sclerotinia* disease on crop plants. *J. Plant Prot. Bull*. [Taiwan] 26: 81–86.

Liu, Sheng Yi, Zhou, Bi Wen, Zhang, Jian Kun, Guo, Xue Lan, Xu, Ze Yong and He, Li Yuan. 2003. Inheritance of resistance to *Sclerotinia sclerotiorum* lesion expansion in rapeseed (*Brassica napus* L.). *Chin. J. Oil Crop Sci*. 25: 54–58.

Liu, Xue Min, Liu, Shu Yan and Xi, Jia Wen. 2002. Studies on the host range of *Sclerotinia sclerotiorum* from *Cleome spinosa. J. Jilin Agril. Univ*. 24: 91–93.

Livingstone, D. M., Hampton, J. L., Phipps, P. M. and Grabau, E. A. 2005. Enhancing resistance to *Sclerotinia minor* in peanut by expressing a barley oxalate oxidase gene. *Plant Physiol*. 137: 1354–1362.

Lobo, M., Jr. and Abreu, M. S. 2000. Inhibition of *Sclerotinia sclerotiorum* mycelial growth by non-volatile metabolites produced by some antagonists under different temperatures and pH's. *Ciencia e Agrotechnologia* 24: 521–526.

Lockhart, C. L. and Delbridge, R. W. 1972. Control of storage diseases of carrots by washing, grading and post-harvest fungicide treatments. *Can. Plant Dis. Surv*. 52: 140–142.

Lockwood, J. L. and Kao, L. B. 1987. Laboratory and field evaluation of soybean for reaction to white mold. *Phytopathology* 77: 1770 (Abstr.).

Lorang, J. M., Tuori, R. P., Martinez, J. P., Sawyer, T. L., Redman, R. S., Rollins, J. A., Wolpert, T. L., Johnson, K. B., Rodriguez, R. J., Dickman, M. B. and Cluffetti, L. M. 2001. Green fluorescent protein is lighting up fungal biology. *Appl. Environ. Microbiol*. 67: 1987–1994.

Loveless, A. R. 1951. Observations on the biology of clover rot. *Ann. Appl. Biol*. 38: 642–664.

Lukashevich, A. I. 1961. Cleaning sunflower seeds from the sclerotia of white rot. *Rev. Appl. Mycol*. 40: 366 (Abstr.).

Lukashevich, A. I. 1964a. Effective control measures against white rot of sunflower. *Rev. Appl. Mycol*. 43: 433 (Abstr.).

Lukashevich, A. I. 1964b. White rot of sunflower and the foundation of methods for its control. *Rev. Appl. Mycol*. 43: 3288.

Lumsden, R. D. 1969. *Sclerotinia sclerotiorum* infection of bean and the production of cellulose. *Phytopathology* 59: 653–657.

Lumsden, R. D. 1970. Phosphatidase of *Sclerotinia sclerotiorum* produced in culture and in infected bean. *Phytopathology* 60: 1106–1110.

Lumsden, R. D. 1972. Localization of pH changes in beans hypocotyls infected with *Sclerotinia sclerotiorum. Phytopathology* 62: 774 (Abstr.).

Lumsden, R. D. 1975. Production in culture of *Sclerotinia sclerotiorum* hyphae similar to infection hyphae in bean. *Proc. Am. Phytopathol. Soc*. 2: 125 (Abstr).

Lumsden, R. D. 1976. Pectolytic enzymes of *Sclerotinia sclerotiorum* and their localization in infected bean. *Can. J. Bot*. 54: 2630–2641.

Lumsden, R. D. 1979. Histology and physiology of pathogenesis in plant disease caused by *Sclerotinia* species. *Phytopathology* 69: 890–896.

Lumsden, R. D. and Dow, R. L. 1973. Histopathology of *Sclerotinia sclerotiorum* infection of bean. *Phytopathology* 63: 708–715.

Lumsden, R. D. and Wergin, W. P. 1980. Scanning electron microscopy of infection of bean by species of *Sclerotinia. Mycologia* 72: 1200–1209

Lumsden, R. D., Lewis, J. A. and Millner, P. D. 1983. Effect of composted sewage sludge on survival soil borne pathogen and diseases. *Phytopathology* 73: 1543–1548.

Lumsden, R. D., Millner, P. D. and Lewis, J. A. 1986. Suppression of lettuce drop caused by *Sclerotinia minor* with composted sewage sludge. *Plant Dis*. 70: 197–201.

Luo, K., Ren, X. G., Zhou, B. W., Chen, D. Y. and Yong, J. 1987. Study of parasitic fungi on sclerotia of *Sclerotinia sclerotiorum* in rape. *Oil crops of China* 3: 40–44.

Luth, P. 2001a. The biological fungicide Contans* WG- a preparation on the basis of the fungus *Coniothyrium minitans*. In: *Proceedings of Sclerotinia 2001, the XI International Sclerotinia Workshop* (C. S. Young and K. J. D. Hughes, eds.). York 8–12 July 2001, Central Science Laboratory, York, England, pp. 127–128.

Luth, P. 2001b. The control of *Sclerotinia* spp. and *Sclerotinia cepivorum* with the biological fungicide Contans* WG- experience from field trails and commercial use. In: *Proceedings Sclerotinia 2001, the XI International Sclerotinia Workshop* (C. S. Young and K. J. D. Hughes, eds.). York 8–12 July 2001, Central Science Laboratory, York, England, pp. 37–38.

Luth, P., Schulz, R. R. and Preffer, H. 1993. The influence of bacterial antagonists on the infestation of a soil as well as on the yield of winter oilseed rape affected by *Sclerotinia sclerotiorum. Zentralbl. Mikrobiol*. 148: 32–38.

Lyons, M. B., Dickson, M. H. and Hunter, J. E. 1987. Recurrent selection for resistance to white mould in *Phaseolus* species. *J. Am. Soc. Hort. Sci*. 112: 149–152.

Maas, A. L., Dashiell, K. E. and Melouk, H. A. 2006. Planting density influences disease incidence and severity of *Sclerotinia* blight in peanut. *Crop Sci*. 46: 1341–1345.

Mackiewicz, S. and Zub, J. 1982. Trials of chemical control of *Sclerotinia sclerotiorum* (Pb.) de Bary on oleaginous sunflower. *Rev. Plant Pathol*. 6: 349 (Abstr.).

Macum, D. B., Grogan, R. G. and Greathead, A. S. 1977. Fungicide control of lettuce drop caused by *Sclerotinia sclerotiorum* and *S. minor. Plant Dis. Reptr*. 61: 555–559.

Madsen, A. M. and Neergaard, E. de. 1999. Interactions between the mycoparasite *Pythium oligandrum* and sclerotia of plant pathogen *Sclerotinia sclerotiorum. Eur. Plant Pathol*. 105: 761–768.

Magro, P., Marciano, P. and Lenna, P. 1984. Oxalic aid production and its role in pathogenesis of *Sclerotinia sclerotiorum. FEMS Microbiol. Lett*. 24: 9–12.

Mahanti, N., Bertrand, H., Monteiro-Vitorella, C. and Fulbright, D. W. 1993. Elevated mitochondrial alternative oxidase activity in ds RNA free, hypovirulent isolates of *Cryphonectria parasitica. Physiol. Mol. Plant Pathol*. 42: 455–463.

Makkonen, R. and Pohjakallio, O. 1960. On the parasites attacking the sclerotia of some fungi pathogenic to higher plants and on the resistance of those sclerotia to their parasites. *Acta. Agric. Scand*. 10: 105–126.

Maltby, A. D. and Mihail, J. D. 1997. Competition among *Sclerotinia sclerotiorum* genotypes within canola stem. *Can. J. Bot*. 75: 462–468.

Manandhar, J. B., Kull, L. S., Mueller, D. S., Hartman, G. L., Pedersen, W. L., Nelson, B. D. (ed.) and Gulya, T. J. 1999. *Sclerotinia sclerotiorum* in soybeans: pathogenic variability, host resist-

ance, and seed infection. In: *Proceedings of the 1998 International Sclerotinia Workshop*, Fargo, ND, 9–12 September, 1998. pp. 36–37.

Marcano, D. A. D., Alvarado, H. G. O. and Abreu, Y. S. 1983. Effect of commercial herbicides on the *in vitro* growth of *Whetzelinia sclerotiorum. Fitopatologia* 18: 25–30.

Marciano, P., Lenna, P. Di and Magro, P. 1983. Oxalic acid, cell wall-degrading enzymes and pH in pathogenesis and their significance in the virulence of two *Sclerotinia sclerotiorum* isolates on sunflower. *Physiol. Mol. Plant Pathol*. 22: 339–345.

Marcum, D. B., Grogan, R. G. and Greathead, A. S. 1977. Fungicide control of lettuce drop caused by *Sclerotinia sclerotiorum* '*minor*.' *Plant Dis. Reptr*. 61: 555–559.

Marinelli, A., March, G. J., Rago, A. and Giuggia, J. 1998. Assessment of crop loss in peanut caused by *Sclerotinia sclerotiorum*, *S. minor* and *Sclerotium rolfsii* in Argentina. *Int. J. Pest Managt*. 44: 251–254.

Marinelli, A., March, G. J., Rago, A. and Giuggia, J. 2004. Effect of environmental conditions on sclerotia germination type of *Sclerotinia sclerotiorum* in peanut crops. *Fitopatologia* 39: 76–83.

Markovic, M. 1992. Chemical control of agents causing early wilt of plants depending on genotype and growth stage of sunflower. *Al Awamia*. 75: 129–159.

Martel, M. B., Herve, Du-Penhoat, C., Letoublon, R. and Fevre, M. 2002. Purification and characterization of a glucoamylases secreted by the plant pathogen *Sclerotinia sclerotiorum. Can. J. Microbiol*. 48: 212–218.

Marukawa, S. and Satamura, Y. 1977. Conditions for reversion of sclerotial formation in a mutant of *Sclerotinia libertiana* Fuckel. *Agric. Biol. Chem*. 41: 655–660.

Marukawa, S., Funakawa, S. and Satomura, Y. 1975a. Some physical and chemical factors on formation of sclerotia in *Sclerotinia libertiana* Fuckel. *Agric. Biol. Chem*. 39: 463–468.

Marukawa, S., Funakawa, S. and Satomura, Y. 1975b. Role of sclerin on morphogenesis in *Sclerotinia sclerotiorum* de Bary (including *S. libertiana* Fuckel). *Agric. Biol. Chem*. 39: 645–650.

Matherson, M. E. and Matejka, J. C. 1989. *In vitro* and field comparison of six new fungicides with iprodione and vinclozolin for control of leaf drop of lettuce caused by *Sclerotinia sclerotiorum. Plant Dis*. 73: 727–730.

Matheron, M. E. and Porchas, M. 2000. First report of stem and crown rot of garbanzo caused by *Sclerotinia minor* in the United States and by *Sclerotinia sclerotiorum* in Arizona. *Plant Dis*. 84: 1250.

Matheron, M. E. and Porchas, M. 2004. Activity of boscalid, fenhexamid, fluazinam, fludioxonil and vinclozolin on growth of *Sclerotinia minor* and *S. sclerotiorum* and development of lettuce drop. *Plant Dis*. 88: 665–668.

Matheron, M. E. and Porchas, M. 2005. Influence of soil temperature and moisture on eruptive germination and viability of sclerotia of *Sclerotinia minor* and *S. sclerotiorum. Plant Dis*. 89: 50–54.

Matheron, M. F. and Matejka, J. C. 1994. First report of *Sclerotinia sclerotiorum* on African daisy in Arizona. *Plant Dis*. 78: 924.

Maxwell, D. P. 1973. Oxalate formation in *Whetzelinia sclerotiorum* by oxalo-acetate acetylhydrolase. *Physiol. Plant Pathol*. 3: 279–288.

Maxwell, D. P. and Lumsden, R. D. 1970.Oxalic acid production by *Sclerotinia sclerotiorum* in infected bean and in culture. *Phytopathology* 60: 1395–1398.

Mazur, D. P. and Grechina, L. F. 1980. Against clover canker. *Zashchita Rostenii*. 10: 42.

McCartney, A., Heran, A. and Li, Q. 2001a. Infection of oilseed rape (*Brassica napus*) by petals containing ascospores of *Sclerotinia sclerotiorum*. In: *Proceedings of Sclerotinia 2001, the XI International Sclerotinia Workshop* (C. S. Young and K. J. D. Hughes, eds.). York 8–12 July 2001, Central Science Laboratory, York, England, pp. 183–184.

McCartney, A., Heran, A., Li, Q. and Freeman, J. 2001b. Petal fall, petal retention and petal duration in oilseed rape crops. In: *Proceedings of Sclerotinia 2001, the XI International Sclerotinia Workshop* (C. S. Young and K. J. D. Hughes, eds.). York 8–12 July 2001, Central Science Laboratory, York, England, pp. 185–186.

McDonald, M. R. and Boland, G. J. 2004. Forecasting diseases caused by *Sclerotinia* spp. in eastern Canada: fact or fiction? *Can. J. Plant Pathol*. 26: 480–488.

McGimpsey, H. C. and Malone, J. P. 1979. Production of apothecia of *Sclerotinia trifoliorum* in the laboratory. *Bull. Br. Mycol. Soc*. 13: 104.

McGimpsey, H. C. and Mercer, P. C. 1984. Sclerotia of *Sclerotinia trifoliorum* in red clover seed (*Trifoliorum pratense*). *Trans. Br. Mycol. Soc*. 82: 567–569.

McLaren, D. L., Huang, H. C., Rimmer, S. R. and Kokko, E. G. 1987. Ultrastructure of the infection of sclerotia of *Sclerotinia sclerotiorum* by *Talaromyces flavus. J. Phytopathol*. 77: 1687 (Abstr).

McLaren, D. L., Huang, H. C., Rimmer, S. R. and Kokko, E. G. 1989. Ultrastructural studies on infection of sclerotia of *Sclerotinia sclerotiorum by Talaromyces flavus. Can. J. Bot*. 67: 2199–2205.

McLaren, D. L, Huang, H. C. and Rimmer, S. R. 1996. Control of apothecial production of *Sclerotinia sclerotiorum* by *Coniothyrium minitans* and *Talaromyces flavus. Plant Dis*. 80: 1373–1378.

McLean, D. M. 1958a. Some experiments concerned with the formation and inhibition of apothecia of *Sclerotinia sclerotiorum* (Lib.) de Bary. *Plant Dis. Reptr*. 42: 409–412.

McLean, D. M. 1958b. Role of dead flower parts in infection of certain crucifers by *Sclerotinia sclerotiorum* (Lib.) de Bary. *Plant Dis. Reptr*. 42: 663–666.

McLoughiin, T. J., Quinn, J. P., Bettermann, A. and Bookland, R. 1992. *Pseudomonas cepacia* suppression of sunflower wilt fungus and role of antifungal compounds in controlling the disease. *Appl. Environ. Microbiol*. 58: 1760–1763.

McMillan, R. T. 1969. Post-harvest control of *Sclerotinia sclerotiorum* of pole bean. *Proc. Florida State Hort. Soc*. 82: 139–140.

McMillan, R. T. 1973. Bean white mold control. *Proc. Florida State Hort. Soc*. 86: 165–166.

McQuilken, M. P. 2001. Integrated control of *Sclerotinia* disease in field grown lettuce in Scotland. In: *Proceedings of Sclerotinia 2001, the XI International Sclerotinia Workshop* (C. S. Young and K. J. D. Hughes, eds.). York 8–12 July 2001, Central Science Laboratory, York, England, pp. 49–50.

McQuilken, M. P. and Whipps, J. M. 1995. Production, survival and evaluation of solid substrate inocula of *Coniothyrium minitans* against *Sclerotinia sclerotiorum. Eur. J. Plant Pathol*. 101: 101–110.

McQuilken, M. P., Metchell, S. J., Budge, S. P., Whipps, J. M., Fenlon, J. S. and Archer, S. A. 1995. Effect of *Coniothyrium minitans* on sclerotial survival and apothecial production of *Sclerotinia sclerotiorum* in field grown oilseed rape. *Plant Pathol*. 44: 883–896.

McQuilken, M. P., Budge, S. P., Whipps, J. M. 1997. Effects of culture media and environmental factors on conidial germination, pycnidial production and hyphal extension of *Coniothyrium minitans. Mycol. Res*. 101: 11–17.

Meinhardt, L. W., Wulff, N. A., Bellato, C. M. and Tsai, S. M. 2002. Telomere and micro satellite primers reveal diversity among *Sclerotinia sclerotiorum* isolates from Brazil. *Fitopatologia Brasileira*. 27: 211–215.

Melouk, A. A., Chanakira, F. A. and Conway, K. E. 1985. Inhibition of *Sclerotinia minor* by *Penicillium citrinum. Phytopathology* 75: 502 (Abstr.)

Melouk, H. A., Singleton, L. L., Oweres, F. N. and Akem, C. N. 1989. Viability of sclerotia of *Sclerotinia sclerotiorum* after passage through the digestive track of a crossbred heifer. *Plant Dis*. 73: 68–69.

Melouk, H. A., Damicone, J. P. and Jackson, K. E. 1992. *Eclipta prostrata* - a new weed host for *Sclerotinia minor. Plant Dis*. 76: 101.

Melouk, H. A., Bowen, C. and Aboshosha, S. S. 1999. A procedure for isolation and deter-mination of incidence of *Sclerotinia minor* in peanut seed. *Alex. J. Agric. Res*. 44: 261–270.

Melzer, M. S. and Boland, G. J. 1994. Epidemiology of lettuce drop caused by *Sclerotinia minor. Can. J. Plant Pathol*. 16: 170–176.

Melzer, M. S. and Boland, G. J. 1996. Transmissible hypo-virulence in *Sclerotinia minor. Can. J. Plant Pathol*. 18: 19–28.

Melzer, M. S., Smith, E. A. and Boland, G. J. 1997. Index of plant hosts of *Sclerotinia minor. Can. J. Plant Pathol*. 19: 272–280.

Melzer, M. S., Ikeda, S. S. and Boland, G. J. 2002. Inter-specific transmission of double-stranded RNA and hypovirulence from *Sclerotinia sclerotiorum* to *S. minor. Phytopathology* 92: 780–784.

Mercier, J. and Reeleder, R. D. 1985. Use of phylloplane fungi to control *Sclerotinia sclerotiorum* on lettuce. *Phytoprotection* 66: 176.

Mercier, J. and Reeleder, R. D. 1987. Interaction between *Sclerotinia sclerotiorum* and other fungi on the phylloplane of lettuce. *Can. J. Bot*. 65: 1633–1637.

Mercier, J., Arul, J., Ponnampalam, R. and Boulet, M. 1993. Induction of 6-methoxymellein and resistance to storage pathogens in carrot slices by UV-C. *J. Phytopathol*. 137: 44–54.

Merriman, P. R. 1976. Survival of sclerotia of *Sclerotinia sclerotiorum* in soil. *Soil Biol. Biochem*. 8: 385–389.

Merriman, P. R., Pywell, M., Harrisen, G. and Nancarrow, J. 1979. Survival of sclerotia of *Sclerotinia sclerotiorum* and effects of cultivation practices on disease. *Soil Biol. Biochem*. 11: 567–570.

Mestries, E., Gentzbittel, L., Labrouhe, D. T. de, Nicolas, P., Vear, F. and De-Labrouhe, D. T. 1998. Analyses of quantitative trait loci associated with resistance to *Sclerotinia sclerotiorum* in sunflowers (*Helianthus annuus* L.) using molecular markers. *Mol. Breed*. 4: 215–226.

Michell, A. J. and Scurfield, G. 1967. Composition of extracted fungal cell walls as indicated by infrared spectroscopy. *Arch. Biochem. Biophys*. 120: 628–637.

Miclaus, D., Sin, G., Damian, V. and Guram, M. 1988. Presence and spread of *Sclerotinia sclerotiorum* (Lib.) de Bary. Seeds of various cereals and industrial crops. I *Analele Institutului de Cercetari Pentru Cereale si Plante Technice, Fundulea* 56: 169–188.

Middleton, K. J., Tatnell, J. and Redelen, R. J. 1995. Germplasm screening of *Phaseolus vulgaris* for resistance to *Sclerotinia sclerotiorum. Aust. Plant Pathol*. 24: 118–125.

Miklas, P. N., Delorme, R., Hannan, R. and Dickson, M. H. 1999. Using a susample of the core collection to identify new sources of resistance to white mold in common bean. *Crop Sci*. 39: 569–573.

Miklas, P. N., Riley, R. H., Grafton, K. F. and Gepts, P. 2001. Quantitative trait loci (QTL) conditioning resistance to white mould in common bean. In: *Proceedings of Sclerotinia 2001, the XI International Sclerotinia Workshop* (C. S. Young and K. J. D. Hughes, eds.). York 8–12 July 2001, Central Science Laboratory, York, England, pp. 59–60.

Miklas, P. N., Delorme, R. and Riley, R. 2003. Identification of QTL conditioning resistance to white mold in snap bean. *J. Am. Soc. Hort. Sci*. 128: 564–570.

Mila, A. L., Carriquiry, A. L., Zhao, J. and Yang, X. B. 2003. Impact of management practices on prevalence of soybean *Sclerotinia* stem rot in the North-Central United States and on farmers' decisions under uncertainty. *Plant Dis*. 87: 1048–1058.

Milenko, Ya. F. 1964. Cleaning of white rot sclerotia from sunflower seed. *Rev. Appl. Mycol*. 43: 542 (Abstr.).

Milinko, I., Fischl, G. and Kadliesko, S. 1989. Data on epidemiology of *Sclerotinia sclerotiorum* (Lib.) de Bary. *Novenytermeles*. 38: 427–434.

Mischke, S., Mischke, C. F. and Adams, P. B. 1995. A rind-associated factor from sclerotia of *Sclerotinia minor* stimulates germination of a mycoparasite. *Mycol. Res*. 99: 1063–1070.

Miller, J. F. and Gulya, T. J. 1999. Registration of eight *sclerotinia*-tolerant sunflower germplasm lines. *Crop Sci*. 39: 301–302.

Miller, J. F. and Gulya, T. J. 2006. Registration of two restorer (RHA 439 and RHA 440) and one maintainer (HA 441) *Sclerotinia* tolerant oilseed sunflower germplasms. *Crop Sci*. 46: 482–483.

Minuto, G., Garibaldi, A., Mocioni, M. and Gullino, M. L. 1999. Chemical control of bottom rot of lettuce caused by *Sclerotinia sclerotiorum. Informatore Fitopatologico* 49: 50–54.

Minuto, A., Pensa, P., Gilardi, G. and Garibaldi, A. 2001. *Sclerotinia sclerotiorum*, a new disease of pot marigold. *Colture Protette*. 30: 95–96.

Minuto, A., Pensa, P., Gullino, M. L. and Garibaldi, A. 2004a. *Sclerotinia sclerotiorum* disease agent of collar rot of *Felicia amelloides* and *Hutchinsia alpina* in Italy. *Informatore Fitopatologico* 54: 40–42.

Minuto, A., Pensa, P., Minuto, G. and Garibaldi, A. 2004b. *Sclerotinia sclerotiorum* agent of stem rot on time (*Thymus citriodorus*) and common sage (*Salvia officinalis*). *Informatore Fitopatologico* 54: 36–39.

Minuto, A., Pensa, P., Rapa, B. and Garibaldi, A. 2005a. Basal and foliar rot of wild rocket [*Diplotaxis tenuifolia* (L.) D.C.] caused by *Sclerotinia sclerotiorum* (Lib.) de Bary in Northern Italy. *Informatore Fitopatologico* 55: 43–45.

Minuto, A., Pensa, P. and Garibaldi, A. 2005b. Attacks of *Sclerotinia sclerotiorum* on *Rosmarinus officinalis* L. var. *prostratus* hort. in Italy. *Informatore Fitopatologico* 55: 50–52.

Mischke, S., Mischke, C. F. and Adams, P. B. 1995. A rind associated factor from sclerotia of *Sclerotinia minor* stimulates germination of a mycoparasite. *Mycol. Res*. 99: 1063–1070.

Mitchell, S. J. and Wheeler, B. E. J. 1990. Factors affecting the production of apothecia and longevity of sclerotia of *Sclerotinia sclerotiorum. Plant Pathol*. 39: 70–76.

Mohan, L. 1994. *Sclerotinia* rot in rosemary. *Indian Phytopath*. 47: 443.

Molinero, Ruiz, M. L. and Melero, Vara, J. M. 2002. First report of stem rot and wilt of sunflower caused by *Sclerotinia minor* in Spain. *Plant Dis*. 86: 697.

Molloy, C., Cheah, Lian Heng and Koolaard, J. P. 2004. Induced resistance against *Sclerotinia sclerotiorum* in carrots treated with enzymatically hydrolyzed chitosan. *Postharvest Biol. Techol*. 33: 61–65.

Mondal, K. K. 2004. Evaluation of seed-dressing fungicides against *Sclerotinia* root rot of buckwheat. *Fagopyrum* 21: 105–107.

Mondal, K. K., Sood, P. and Rana, S. S. 2003. Occurrence of *Sclerotinia sclerotiorum* on buckwheat (*Fagopyrum esculentum*) seedlings in Himachal Pradesh. *Plant Dis. Res*. 18: 199.

Mondolot-Cosson, L., Andary, C., Geibel, M., Treutter, D. and Feucht, W. 1994. Resistance factor of a wild species of sunflower *Helianthus resinosus* to *Sclerotinia sclerotiorum. Acta Hort*. 381: 642–645.

Moons, B. 1986. Pesticide research report. *Expert. Comm. Pesticide use Agric*. [Canada]: 212.

Moore, W. D. 1949. Flooding as a means of destroying sclerotia of *Sclerotinia sclerotiorum. Phytopathology* 39: 920–927.

Moore, W. D. 1955. Relation of rainfall and temperature to the incidence of *Sclerotinia sclerotiorum* in vegetables in South Florida during the years 1944 to 1954. *Plant Dis. Reptr*. 39: 470–472.

Moore, W. D., Conover, R. A. and Stoddard, D. L. 1949. The *Sclerotinia* disease of vegetable crops in Florida. *Fla. Agr. Exp. Sta. Press. Bull*. 457: 20.

Mordue, J. E. M. 1998a. *Sclerotinia fructicola*, IMI Descriptions of Fungi and Bacteria. (62) Sheet 616, CABI Bioscience, Bakeham Lane, Egham, Surrey, TW20 9TY, UK.

Mordue, J. E. M. 1998b. *Sclerotinia fructigena,* IMI Descriptions of Fungi and Bacteria. (62) Sheet 617, CABI Bioscience, Bakeham Lane, Egham, Surrey, TW20 9TY, UK.

Mordue, J. E. M. 1998c. *Sclerotinia homoeocarpa,* IMI Descriptions of Fungi and Bacteria. (62) Sheet 618, CABI Bioscience, Bakeham Lane, Egham, Surrey, TW20 9TY, UK.

Mordue, J. E. M. 1998d. *Sclerotinia laxa,* IMI Descriptions of Fungi and Bacteria. (62) Sheet 619, CABI Bioscience, Bakeham Lane, Egham, Surrey, TW20 9TY, UK.

Mordue, J. E. M. and Holliday, P. 1998. *Sclerotinia sclerotiorum,* IMI Descriptions of Fungi and Bacteria. (52) Sheet 513, CABI Bioscience, Bakeham Lane, Egham, Surrey, TW20 9TY, UK.

Morrall, R. A. A. 1977. A preliminary study of the influence of water potential on sclerotium germination in *Sclerotinia sclerotiorum. Can. J. Bot*. 55: 8–11.

Morrall, R. A. A. and Dueck, J. 1982. Epidemiology of *Sclerotinia* stem rot of rapeseed in Saskatchewan. *Can. J. Plant Pathol*. 4: 161–168.

Morrall, R. A. A. and Dueck, J. 1983. *Sclerotinia* stem rot of spring rapeseed in Western Canada. *Proceedings of the 6th International Rapeseed Conference*, *Paris* [France]: 957–962 (Abstr.).

Morrall, R. A. A., Duczek, I. J. and Sheard, J. W. 1972. Variation and correlation within and between morphology, pathogenicity and pectolytic enzyme activity in *Sclerotinia* from Saskatchewan. *Can. J. Bot*. 50: 767–786.

Morrall, R. A. A. and Duczek, I. J., Mc Kenzie, D. L. and McGee, D. C. 1976. Some aspects of *Sclerotinia sclerotiorum* in Saskatchewan. 1970–75. *Can. Plant Dis. Surv*. 56: 56.

Morrall, R. A. A., Loew, F. M. and Hayes, M. A. 1978. Subacute toxicological evaluation of sclerotia of *Sclerotinia sclerotiorum* in rats. *Can. J. Compo Med*. 42: 473–477.

Morrall, R. A. A., Royers, R. R. and Rude, S. Y. 1989. Improved techniques of controlling *Sclerotinia* stem rot of canola (oilseed rape) with fungicides in Western Canada. *Mededelingenvan de Facultert Landbouwwatens Chappen Rijksuniversiteit Gent*. 54: 643–649.

Mortensen, K. and Hogue, E. J. 1995. *Sclerotinia sclerotiorum* as a potential biological control agent for diffuse knapweed on dry rangland in interior British Columbia. In: *Proceedings of VIII International Symposium of Biological Control of Weeds, 1992*. Lincoln Univ. Canterbury, New Zealand (E. S. Delfosse and R. R. Scott, eds.), DSIR/CSIRO, Melbourne, Australia, pp. 397–406.

Morton, J. G. and Hall, R. 1989. Factors determining the efficiency of chemical control of white mold in white bean. *Can. J. Plant Pathol*. 11: 297–302.

Mosa, A. A., Abd Allah, S. M. and Ibrahim, M. M. 2000a. Factors affecting germination of sclerotia of *Sclerotinia sclerotiorum. Ann. Agric. Sci. Cairo* 4(Special): 1353–1371.

Mosa, A. A., El Deeb, A. A. and Ibrahim, M. M. 2000b. Evaluation of sunflower hybrids for resistance to *Sclerotinia sclerotiorum. Ann. Agric. Sci. Cairo* 45: 689–702.

Mouly, A., Rumeau, D. and Esquerre-Tagaye, M. T. 1990. Markers of *Sclerotinia* tolerance for breeding sunflower. *Mol. Markers Bull. CETIOM*. 106: 8.

Muckel, R. O. and Steadman, J. R. 1981. Dissemination and survival of *Sclerotinia sclerotiorum* in bean field in Western Nebraska. *Phytopathology* 71: 244.

Mueller, D. S., Hartman, G. L. and Pedersen, W. L. 1999. Development of sclerotia and apothecia of *Sclerotinia sclerotiorum* from infected soybean seed and its control by fungicide seed treatment. *Plant Dis*. 83: 1113–1115.

Mueller, D. S., Dorrance, A. E., Derksen, R. C., Ozkan, E., Kurle, J. E., Grau, C. R., Gaska, J. M., Hartman, G. L., Bradley, C. A. and Pedersen, W. L. 2002. Efficacy of fungicides on *Sclerotinia sclerotiorum* and their potential for control of *Sclerotinia* stem rot on soybean. *Plant Dis*. 86: 26–31.

Mueller, D. S., Bradley, C. A., Grau, C. R., Gaska, J. M., Kurle, J. E. and Pedersen, W. L. 2004. Application of thiophanate-methyl at different host growth stages for management of *Sclerotinia* stem rot in soybean. *Crop Prot*. 23: 983–988.

Mueller, D. S., Hartman, G. L. and Pedersen, W. L. 2005. Effect of crop rotation and tillage system on *Sclerotinia* stem rot on soybean. *Can. J. Plant Pathol*. 24: 450–456.

Mukula, J. 1957. On the decay of stored carrots in Finland. *Acta Agric. Scand. Suppl* 2.

Mullins, E., Quinlan, C. and Jones, P. 1995. Analysis of mechanism of partial physiological resistance to *Sclerotinia sclerotiorum* using induced mutants of *Brassica napus. Aspects Appl. Biol*. 42: 307–314.

Mullins, E., Quinlan, C. and Jones, P. 1999. Isolation of mutants exhibiting altered resistance to *Sclerotinia sclerotiorum* from small M_2 populations of an oilseed rape (*Brassica napus*) variety. *Eur. J. Plant Pathol*. 105: 465–475.

Mundel, H. H., Huang, H. C., Braun, J. P. and Kiekn, F. A. 1996. Registration of AC sunset safflower. *Crop Sci*. 36: 804–805.

Myers, J. R., Stewart-Williams, K. D., Hayers, R. E., Lancaster, M. W. and Kolar, J. J. 1997. Registration of UI-911 black bean and UI-137 of Navy bean. *Crop Sci*. 37: 285–286.

Mylehreest, S. J. and Wheeler, R. E. J. 1987. A method for inducing apothecia from sclerotia of *Sclerotinia sclerotiorum. Plant Pathol*. 36: 16–20.

Naar, Z. and Kecskes, M. 1997. Combined effect of *Trichoderma viride* and vinclozolin on the viability of *Sclerotinia minor* sclerotia. *Novenyvedelem* 33: 173–179.

Nagy, P. and Fischi, G. 2002. Effect of UV and visible light irradiation on mycelial growth and sclerotium formation of *Sclerotinia sclerotiorum. Acta Phytopathologica et Entomologica Hungarica* 37: 83–89.

Naito, N. and Tani, T. 1952. Effect of 2, 4-D in culture media on mycelial growth, sporulation and sclerotia formation by various phytopathogenic fungi. *Tech. Bull. Kagiwa. Agric. Coll*. 4: 50–55.

Nakashima, N., Moromizato, Z. and Matsuyama, N. 1991. *Chaetomium* spp., antagonistic microorganism to phypathogenic fungi. *J. Fac. Agril. Kyushu Univ*. 36: 109–115.

Nannfeldt, J. A. 1976. Iodine reaction in asens plugs and their taxonomic significance. *Trans. Br. Mycol. Soc*. 67: 283–287.

Narumi, T., Fujita, T., Sano, T. and Harada, Y. 2001. *Sclerotinia trillii*, sp. *nov*., causing a new *Sclerotinia* disease on *Trillium* in Japan. *Mycoscience* 42: 181–186.

Nath, S., Das, B. C. and Sarmah, D. K. 2004. Management of white mold (*Sclerotinia sclerotiorum*) of french bean (*Phaseolus vulgaris*) with carrier based formulation of *Trichoderma harzianum. Indian J. Agric. Sci*. 74: 168–170.

Natti, J. J. 1967. Bean white mold control with foliar sprays. *Farm Res*. 33: 10–11.

Natti, J. J. 1971. Epidemiology and control of bean white mold. *Phytopathology* 61: 669674.

Naumova, N. A. 1957. Peculiarities in the development of *Sclerotinia* on sunflower under the condition of Voronzh region. *Rev. Appl. Mycol*. 36: 765 (Abstr.).

Nedeleu, L., Alexandri, A. A. and Baicu, T. 1988. Pathogenicity of some isolates of *Sclerotinia sclerotiorum* and their behavior towards some fungicides. *Probleme de Protectia Plantelor* 16: 267–274.

Neergaard, P. 1958. Mycelial seed infection of certain crucifers by *Sclerotinia sclerotiorum* (Lib.) de Bary. *Plant Dis. Reptr*. 42: 1105–1106.

Neklyudova, E. T. and Parko, N. M. 1981. Evaluation of the resistance to *Helianthus tuberosum* to *Sclerotinia. Rev. Plant Pathol*. 60: 2916 (Abstr.).

Nelson, B. D. 1985a. A seedling test to evaluate virulence of *Sclerotinia sclerotiorum* isolated in sunflower. *Phytopathology* 75: 1333 (Abstr.).

Nelson, B. D. 1985b. Inoculation preparation of *Sclerotinia sclerotiorum* for infection of sunflower and other hosts. *Phytopathology* 75: 1333 (Abstr).

Nelson, B. D., Hertgaard, D. M. and Holley, R. C. 1989. Disease progress of *Sclerotinia* wilt of sunflower at varying plant populations, inoculum densities and environments. *Phytopathology* 79: 1358–1368.

Nelson, B. D., Helms, T. C. and Kural, I. 1991a. Effect of temperature and pathogens isolates on laboratory screening of soybean for resistance to *Sclerotinia sclerotiorum. Can. J. Plant Sci*. 71: 347–352.

Nelson, B. D., Helms, T. C. and Olson, M. A. 1991b. Comparison of laboratory and field evaluations of resistance in soybean to *Sclerotinia sclerotiorum. Plant Dis*. 75: 662–665.

Nelson, B. D., Christianson, T. and Mc Clean, P. 2001. Effects of bacteria on sclerotia of *Sclerotinia sclerotiorum*. In: *Proceedings of Sclerotinia 2001, the XI International Sclerotinia Workshop* (C. S. Young and K. J. D. Hughes, eds.). York 8–12 July 2001, Central Science Laboratory, York, England, pp. 39–40.

Nelson, E. B. and Craft, C. M. 1991. Introduction and establishment of strains of *Enterobacter cloacae* in golf course turf for the biological control of dollar spot. *Plant Dis*. 75: 510–514.

Nelson, K. A., Renner, K. A. and Hammerschmidt, R. 2002a. Effects of protoporphyrinogen oxidase inhibitors on soybean (*Glycine max* L.) response, *Sclerotinia sclerotiorum* disease development and phytoalexins production by soybean. *Weed Technol*. 16: 353–359.

Nelson, K. A., Renner, K. A. and Hammerschmidt, R. 2002b. Cultivar and herbicide selection affects soybean development and the incidence of *Sclerotinia* stem rot. *Agron. J*. 94: 1270–1281.

Newton, H. K. 1972. *Sclerotinia sclerotiorum* an incitant of lettuce drop: sources of inoculum, host resistance and mechanism of pathogenesis. Ph.D. thesis, Murofolm 72-V.292, University of Murofilm Inc., Ann Arbor, MI, 135 pp.

Newton, H. K. and Sequeira, L. 1972a. Ascospores as the primary infective propagules of *Sclerotinia sclerotiorum* in Wisconsin. *Plant Dis. Reptr*. 56: 798–802.

Newton, C. H. and Sequeira, L. 1972b. Possible sources of resistance in lettuce to *Sclerotinia sclerotiorum. Plant Dis. Raptr*. 56: 875–878.

Newton, H. K., Maxwell, D. P. and Sequeira, L. 1973. Conductivity assay for measuring virulence of *Sclerotinia sclerotiorum. Phytopathology* 63: 424–428.

Nicholson, J. F., Dhingra, O. D. and Sinclair, J. B. 1972. Internal seed-borne nature of *Sclerotinia sclerotiorum* and *Phomopsis* spp. and their effects on soybean seed quality. *Phytopathology* 62: 1261–1263.

Nicot, M. J. 1960. Some characteristics of the microflora in desert sand. In: *The Ecology of Soil Fungi* (D. Parkinson and J. S. Waid, eds.). Liverpool University Press, Aylesbury, pp. 94–97.

Nilsson, H. E. 1985. Remote sensing of oilseed rape infected by *Sclerotinia* stem rot and Verticillium wilt. *Sverigas Lautbrunksunivirsitet, Uppsala.*: 33.

Nisikado, Y. and Hirata, K. 1937. Studies on the longevity of sclerotia of certain fungi under controlled environmental factors. *Ber. Ohara Inst.* VII, 4: 535–547.

Nobel, M. and Richardson, M. J. 1968. *An Annotated List of Seed Borne Diseases*. Common Wealth Mycological Institute, Kew, Surrey, England, 191 pp.

Noon, R. A., Northwood, P. J., Brown, M. C., Montury, A. and Charlet, C. 1988. Flutrafol based formulation for control of oilseed rape diseases. *Proc. Br. Crop. Prot. Conf. Pest Dis.* 3: 947–952.

Noonan, M. P., Glare, T. R., Harvey, I. C., Sands, D. C. and O' Callaghan, M. 1996. Genetic comparison of *Sclerotinia sclerotiorum* isolates from New Zealand and USA. *Proceedings of Forty Ninth New Zealand Plant Protection Conference*, New Zealand, 13–15 August, 1996, pp. 126–131.

Nordin, K., Sigvald, R. and Svensson, C. 1992. Forecasting the incidence of *Sclerotinia* rot on spring sown rapeseed. *Zeitchrift fur Planzenkrankheiten und Pflanzenschutz*. 99: 245–255.

Noviello, C. and Korf, R. P. 1961. A simple technique for investing stomatal formation in the *Sclerotinia*ceae. *Mycologia* 37: 648–714.

Noyes, R. D. and Hancock, J. G. 1981. Role of oxalic acid in the *Sclerotinia* wilt of sunflower. *Physiol. Plant Pathol.* 8: 123–132.

Nuss, D. L. and Koltin, Y. 1990. Significance of dsRNA genetic elements in plant pathogenic fungi. *Ann. Rev. Phytopathol.* 28: 37–58.

Oedjijono, M. A. L. and Dragar, C. 1993. Isolation of bacteria antagonistic to a range of plant pathogenic fungi. *Soil Biol. Biochem.* 25: 247–250.

Oliveira, S. H. F., Recco, C. A., Sugahara, E. and Oliveira, D. A. 1995. Comparative evaluation of fumigation and conventional spray of fungicides to bean *Sclerotinia sclerotiorum* control. *Summa Phytopathologica* 21: 249–252.

Olsson, K. and Svensson, R. 1996. The influence of polyacetylenes on the susceptibility of carrots to storage diseases. *J. Phytopathol.* 144: 441–447.

Orellana, R. G. 1975. Photoperiod influence on the susceptibility of sunflower to *Sclerotinia* stalk rot. *Phytopathology* 65: 1293–1298.

Orellana, R. G., Foy, C. D. and Fleming, A. L. 1975. Effect of soluble aluminium on growth and pathogenicity of *Verticillium alboatrum* and *Whetzelinia sclerotiorum* from sunflower. *Phytopathology* 65: 202–205.

Overell, B.T. 1952. A toxin in culture filtrates of *Sclerotinia sclerotiorum. Austr. J. Sci.* 14: 197–198.

Pan-Hong, Yu, Xi, H. J., Liu, W. C., Wang, Q. and Li, H. Yu. 2001. Optimal stage and threshold of the control of *Sclerotinia* rot. *Acta Phytophylacica Sinica* 28: 299–302.

Pan, Yi Lou, Wang, Zhi Yuan, and Wu, Han Zhang 1997. Resistance to carbendazim and stability of the resistance in *Sclerotinia sclerotiorum. Jiangsu J. Agric. Sci.* 13: 32–35.

Pan, Yi Lou, Liu, Fu Hai and Xu, Zhi Ping. 2000. The resistance of *Sclerotinia sclerotiorum* of rape to carbendazim and its management. *Jiangsu Agril. Sci.* 3: 39–40.

Pandey, K. K. and Pandey, P. K. 2002. A new report of *Sclerotinia sclerotiorum* on bottle gourd. *Indian Phytopath.* 55: 352–353.

Pandey, R. N. and Saharan, G. S. 2005. Facultative fungal diseases of sunflower. In: *Diseases of Oilseed Crops* (G. S. Saharan, N. Mehta, M. S. Sangwan, eds.). Indus Publishing, New Delhi, pp. 373–459.

Parfitt, D., Coley- Smith, J.R. and Jeves, T.M. 1983. *Teratosperma oligocladium*, a mycoparasite of fungal sclerotia. *Plant Path.* 32: 459–460.

Park, S. K., Lee, H. Y. and Kim, S. C. 1995. Antagonistic effect of chitinolytic bacteria on soil borne plant pathogens. *Kor. J. Plant Pathol.* 11: 47–52.

Partyka, R. E. and Mai, W. F. 1962. Effect of environment and some chemicals on *Sclerotinia sclerotiorum* in laboratory and potato field. *Phytopathology* 52: 766–770.

Pathak, A. K., Godika, S., Jain, J. P. and Muralia, S. 2001. Effect of antagonistic fungi and seed dressing fungicides on the incidence of stem rot of mustard. *J. Mycol. Plant Pathol.* 31: 327–329.

Pathak, A. K., Godika, S., Jain, J. P. and Muralia, S. 2002. Screening of *Brassica* genotypes against stem rot disease of mustard caused by *Sclerotinia sclerotiorum* (Lib.) de Bary. *J. Mycol. Plant Pathol.* 32: 111–112.

Patterson, C. L. 1986. The comparative biology, epidemiology and control of lettuce drop caused by *Sclerotinia minor* and *S. sclerotiorum* and the genetic analysis of vegetative and sexual compatibility in *S. minor*. Ph.D. thesis, University of California, Davis, 76 p.

Patterson, C. L. and Grogan, R. G. 1985. Differences in epidemiology and control of lettuce drop caused by *Sclerotinia minor* and *S. sclerotiorum. Plant Dis.* 69: 766–770.

Paul, V. H. and Beineke, M. 1993. Reaction of cultivars and effect of fungicides on winter oilseed rape diseases in 1990–91. *Bull. OILB-SROP* 16: 124–135.

Paul, Y. S. 1996. Status of *Sclerotinia sclerotiorum*-a polyphagous pathogen in Lahaul valley of Himachal Pradesh. *Plant Dis. Res.* 11: 172–174.

Paulitz, T. C. 1997. First report of *Sclerotinia sclerotiorum* on basil in Canada. *Plant Dis.* 81: 229.

Pawlowski, S. H. and Hawn, E. J. 1964. Host–parasite relationship in sunflower wilt incited by *Sclerotinia sclerotiorum* as determined by the twin technique. *Phytopathology* 54: 33–35.

Pedras, M. S. C. and Ahiahonu, P. W. K. 2004. Phytotoxin production and phytoalexin elicitation by the phytopathogenic fungus *Sclerotinia sclerotiorum. J. Chem. Eco.* 30: 2163–2179.

Pelmus, A., Craiciu, M., Penesiu, A. and Popov, C. 1988. Effect of some herbicides on the pathogen *Sclerotinia sclerotiorum* Lib. de Bary. *Probleme de Protectia Plantelor* 16: 147–159.

Peng, M. and Kuc, J. 1992. Peroxidase generated hydrogen peroxide as a source of antifungal activity *in vitro* and on tobacco leaf disks. *Phytopathology* 82: 696–699.

Pereira, J. C. R., Chaves, G. M., Zambolim, L., Matsuoka, K., Silva-Acuna, R. and Vale, F. X. R. do. 1996. Integrated control of *Sclerotinia sclerotiorum. Fitopatologia Brasileira* 21: 254–260.

Peres, A. P., Nasser, L. C. B. and Machado, J. C. da 2002. Use of semi-selective media for detection of *Sclerotinia sclerotiorum* on bean and soybean seeds. *Fitopatologia Brasileira* 27: 123–127.

Perez, A., Regnault, Y. and Allard, L. M. 1989. *Sclerotinia sclerotiorum*, effect of five agronomic factors on the intensity of attacks on sunflower. *Information Techniques CETIOM* 107: 7–13.

Perone, V. B., Scheel, L. D. and Meitus, R. J. 1964. A bioassay for the quantification of cutaneous reactions associated with pink-rot celery. *J. Invest. Dermatal.* 42: 267–271.

Peterson, G. R., Russo, G. M. and van Etten, J. L. 1982. Identification of major proteins in sclerotia of *Sclerotinia minor* and *Sclerotinia trifoliorum. Exp. Mycol.* 6: 268–273.

Peyen, D. 1983. Modelling the epidemiology of *Sclerotinia sclerotiorum* on sunflower. *Bull. OEPP* 13: 277–281.

Phillips, A. J. L. 1986a. Factors affecting the parasitic activity of *Gliocladium virens* on sclerotia of *Sclerotinia sclerotiorum* and a note on its host range. *J. Phytopathol.* 116: 212–220.

Phillips, A. J. L. 1986b. Carpogenic germination of sclerotia of *Sclerotinia sclerotiorum* after periods of conditioning in soil. *J. Phytopathol.* 116: 247–258.

Phillips, A. J. L. 1990. The effect of soil solarization on sclerotial population of *Sclerotinia sclerotiorum. Plant Pathol.* 39: 38–43.

Phillips, A. J. L. 1992. Some common weed spp. as alternative host for *Sclerotinia sclerotiorum. Phytophylactica* 24: 207–210.

Phillips, A. J. L. and Price, K. 1983. Structural aspects of the parasitism of sclerotia of *Sclerotinia sclerotiorum* (Lib.) de Bary by *Coniothyrium minitans* Campb. *Phytopath. Z.* 107: 193–203.

Phillips, N. M., Grafton, K. F. and Phillip, E. M. 1993. Estimating phenylalanine ammonialyase activity in common bean inoculated with *Sclerotinia sclerotiorum. Hort. Sci.* 28: 937–938.

Phipps, P. M. and Porter, D. M. 1982. *Sclerotinia* blight of soybean caused by *Sclerotinia minor* and *Sclerotinia sclerotiorum. Plant Dis.* 66: 163–165.

Pierre, J. G., Charlet, C. D., Brun, H., Penaual, A., Vaysse, C., Lacombe, J. P., Martin, D., Regnault, Y., Labrouhe, D. T. and Sarrazin, J. F. Techniques for the artificial contamination of oilseed rape in trial plots. *CETIOM*: 26–33.

Pierson, P. E. and Rhodes, L. H. 1992. Effect of culture medium on the production of oxalic acid by *Sclerotinia trifoliorum. Mycologia* 84: 467–469.

Pieta, D. 1990. Susceptibility of french bean to fungal infection in relation to chemical composition of the leaves and root exudates. *Hodowla Roslin Aklimatyzacja I Nasinnictwo* 34: 53–61.

Pieta, D. 1994. Biochemical factors conditioning resistance in French bean to infection with pathogenic fungi. *Biuletyn Warzywniczy* 41: 117–122.

Pinto, C. M. F., Maffia, L. A., Casali, V. W. D. and Cardoso, A. A. 1998. *In vitro* effect of plant extracts on mycelial growth and sclerotial germination of *Sclerotinia sclerotiorum. J. Phytopath*. 146: 421–425.

Pirvu, N., Vranceanu, A. V. and Stolenescu, F. 1985. Genetic mechanism of sunflower resistance to white rot (*Sclerotinia sclerotiorum*) Lib. de Bary. *Z. Pflanzenziichtg* 95: 157–163.

Podkina, D. V., Kotlyarova, L. A. and Sukhareva, O. N. 1988. Methods of evaluating breeding material of soybean for resistance to *Fusarium* and *Sclerotinia. Nauchno-Tekhnicheskii Byulleten Vsesoyuznogo Nauchno-Issledovatel Skogo. Instituta Maslichnykh Kultur*: 21–23.

Pohjakallio, O. and Salomen, A. 1950. On the appearance and significance of fungi and bacteria antibiotically affecting the fungus *Sclerotinia trifoliorum* Erikss. *Maatalous Aikak*. 22: 63–67.

Pohjakallio, O., Salomen, A. and Lackkonen, E. 1949. Investigations into the bacteria antibiotically affecting the fungus *Sclerotinia trifoliorum* Erikss. *Physiol. Mol. Plant Pathol*. 2: 312–322.

Polyakov, P. 1973. White rot of sunflower caused by *Sclerotinia sclerotiorum* (de Bary) Fekl. *Rev. Plant Pathol*. 52: 473 (Abstr.).

Pope, S. J. and Sweet, J. B. 1991. *Sclerotinia* stem rot resistance in linseed cultivars. *Aspect Appl. Biol*. 28: 78–84.

Porter, D. M. 1977. The effect of chlorothalonil and benomyl on the severity of *Sclerotinia* blight of peanut. *Plant Dis. Reptr*. 61: 995.

Porter, D. M. 1980a. *Sclerotinia* blight of groundnut. A disease of major importance in the USA. In: *Proceedings of International Workshop on Groundnut* (R. W. Gibbons and J. V. Martin, eds.). ICRISAT, Patancheru, India, 177.

Porter, D. M. 1980b. Increased severity of *Sclerotinia* blight in peanut treated with captafol and chlorothalonil. *Plant Dis*. 64: 394.

Porter, D. M. 1980c. Control of *Sclerotinia* blight of peanut with procymidone. *Plant Dis*. 64: 865.

Porter, D. M. 1984. *Sclerotinia* blight. In: *Compendium of Peanut Diseases* (D. M. Porter, D. H. Smith and R. Rodriguez Kabana, eds.). American Phytopathological Society, St. Paul, MN, pp. 16–18.

Porter, D. M. and Beute, M. K. 1974. *Sclerotinia* blight of peanut. *Phytopathology* 64: 263–264.

Porter, D. M. and Lankow, R. K. 1981. Growth of *Sclerotinia minor* on media containing chlorothalonil and benomyl. *Plant Dis*. 65: 591.

Porter, D. M. and Powell, N. L. 1978. *Sclerotinia* blight development of peanut vines injured by tractor tires. *Peanut Sci*. 5: 87.

Porter, D. M. and Rud, O. E. 1980. Suppression of *Sclerotinia* blight of peanut with dinitrophenol herbicides. *Phytopathology* 70: 720–722.

Porter, D. M. and Steele, J. L. 1983. Quantitative assay by elutriation of peanut field soil for sclerotia of *Sclerotinia minor. Phytopathology* 73: 636–640.

Porter, D. M., Beute, M. K. and Wynne, J. C. 1975. Resistance of peanut germplasm to *Sclerotinia sclerotiorum. Peanut Sci*. 2: 78–80.

Porter, D. M., Powell, N. L. and Cobb, P. R. 1977. Severity of *Sclerotinia minor* blight of peanuts as detected by infrared aerial photography. *Peanut Sci*. 4: 75.

Porter, D. M., Coffelt, T. A., Wright, F. S. and Mozingo, R. W. 1992. Resistance to *Sclerotinia* blight and early leaf spot in Chinese peanut germplasm. *Plant Sci*. 19: 41–43.

Pottinger, B. M., Ridgway, H. J., Bourdot, G. and Stewart, A. 2004. Abiotic factors affecting the pathogenicity of a *Sclerotinia sclerotiorum* based mycoherbicide. In: *Proceedings of 14th*

Australian Weeds Conference, Wagga. New South Wales, Australia, 6–9 September, 2004, pp. 345–348 (Abstr.).

Pound, G. S. 1946. Diseases of cabbage plants grown for seeds in western Washington. *Int. Agric. Sci. Agric. Exp. Sta. Pullman*, Washington. *Bull*. No. 475: 27pp.

Poussereau, N., Creton, S., Billon-Grand, G., Rascle, C. and Fevre, M. 2001a. Regulation of *acp1* encoding a non-aspatyl acid protease expressed during pathogenesis of *Sclerotinia sclerotiorum. Microbiology* 147: 717–726.

Poussereau, N., Gente, S., Rascle, C., Billon-Grand, G. and Fevre, M. 2001b. *aspS* encoding an unusual aspartyl protease from *Sclerotinia sclerotiorum* is expressed during phytopathogenesis. *FEMS Microbiol. Lett*. 194: 27–32.

Powell, N. L., Porter, D. M. and Pettry, D. E. 1976. Use of aerial photography to detect diseases in peanut field I *Sclerotinia* blight. *Peanut Sci*. 3: 21–24.

Prajapati, C. R., Shukla, H. P. and Pandey, R. 2005. Screening of *Dolichos* bean cultivars/germplasms against *Sclerotinia sclerotiorum. Ann. Plant Prot. Sci*. 13: 259–261.

Prats, E., Bazzalo, M. E., Leon, A. and Jorrin, J. V. 2003. Accumulation of soluble phenolic compounds in sunflower capitula correlates with resistance to *Sclerotinia sclerotiorum. Euphytica* 132: 321–329.

Price, K. and Colhoun, J. 1975a. A study of variability of isolates of *Sclerotinia sclerotiorum* (Lib.) de Bary from different hosts. *Phytopathol. Z.* 83: 159–166.

Price, K. and Colhoun, J. 1975b. Pathogenicity of isolates of *Sclerotinia sclerotiorum* (Lib.) de Bary to several hosts. *Phytopathol. Z.* 83: 232–238.

Prior, G. D. and Owen, J. H. 1964. Pathological anatomy of *Sclerotinia trifoliorum* on clover and alfalfa. *Phytopathology* 54: 784–787.

Pratt, R. G. 1996. Screening for resistance to *Sclerotinia trifoliorum* in alfalfa by inoculation of excised leaf tissue. *Phytopathology* 86: 923–928.

Pratt, R. G. and Knight, W. E. 1982. Formation of apothecia by sclerotia of *Sclerotinia sclerotiorum* and infection of crimson clover in the field. *Plant Dis*. 66: 1021–1023.

Pratt, R. G. and Rowe, D. E. 1995. Comparative pathogenicity of isolates of *Sclerotinia trifoliorum* and *S. sclerotiorum* on alfalfa cultivars. *Plant Dis*. 79: 474–477.

Pratt, R. G. and Rowe, D. E. 1998. Evaluation of simplified leaf inoculation procedures for identification of quantitative resistance to *Sclerotinia trifoliorum* in alfalfa seedlings. *Plant Dis*. 82: 1161–1164.

Pritchard, M. K., Boese, D. E. and Rimmer, S. R. 1992. Rapid cooling and field applied fungicides for reducing losses in stored carrots caused by cottony soft rot. *Can. J. Plant Pathol*. 14: 177–181.

Pszczola, J. 1994. Bor-a new double-zero winter swede rape variety. *Oilseed Crops. XVIth Polish Research Conference*. April, 19–20, 1994. *Rosliny-Oleiste* 15: 167–169.

Purdy, L. H. 1955. A broader concept of the species *Sclerotinia sclerotiorum* based on variability. *Phytopathology* 45: 421–427.

Purdy, L. H. 1956. Factors affecting apothecial production by *Sclerotinia sclerotiorum. Phytopathology* 46: 409–410.

Purdy, L. H. 1958. Some factors affecting penetration and infection by *Sclerotinia sclerotiorum. Phytopathology* 48: 605–609.

Purdy, L. H. 1979. *Sclerotinia sclerotiorum:* History, diseases and symptomatology, host range, geographical distribution and impact. *Phytopathology* 69: 875–880.

Purdy, L. H. and Bardin, R. 1953. Mode of infection of tomato plant by the ascospores of *Sclerotinia sclerotiorum. Plant Dis. Reptr*. 37: 361–362.

Purdy, L. H. and Grogan, R. L. 1954. Physiological studies of *Sclerotinia sclerotiorum* in liquid and agar culture. *Phytopathology* 44: 36–38.

Quentin, U. 2004. *Sclerotinia sclerotiorum*, occurrence and control. *Kartoffelbau* 8: 318–319.

Rader, W. E. 1952. Diseases of stored carrots in New York State. *N.Y. Agric Exp. Stn. Geneva Bull*. 889: 10–14.

Radke, V. L. and Grau, C. R. 1982. Effect of herbicides on carpogenic germination of *Sclerotinia sclerotiorum. Phytopathology* 72: 1139 (Abstr.).

Radke, V. L. and Grau, C. R. 1986. Effects of herbicides on carpogenic germination of *Sclerotinia sclerotiorum. Plant Dis*. 70: 19–23.
Rai, J. N. and Dhawan, S. 1976a. Production of polymethyl galacturonase and cellulose and its relationship with virulence in isolates of *Sclerotinia sclerotiorum* (Lib.) de Bary. *Indian J. Exp. Biol*. 14: 197.
Rai, J. N. and Dhawan, S. 1976b. Studies on purification on identification of toxic metabolite produced by *Sclerotinia sclerotiorum* causing white rot disease of crucifers. *Indian Phytopath*. 29: 407–411.
Rai, J. N. and Saxena, V. C. 1975. Sclerotial mycoflora and its role in natural biological control of white rot disease. *Plant Soil* 43: 509–513.
Rai, J. N. and Dhawan, S. 1978. Lysis of sclerotia of *Sclerotinia sclerotiorum* and its possible relation with chitinase activity. *Indian J. Mycol. Plant Pathol*. 8: 103–107.
Rai, J. N., Singh, R. P. and Saxena, V. C. 1979. Phenolics and cell wall degrading enzymes in *Sclerotinia* infection of resistant and susceptible plants of *Brassica juncea. Indian J. Mycol. Pl. Pathol*. 9: 160–165.
Rajappan, K., Ramaraj, B. and Natarajan, S. 1999. Stem rot of *Salvia coccinea* caused by *Sclerotinia sclerotiorum* - a new host record. *Indian Phytopath*. 52: 425.
Ralph, R. K. 1969. Double-stranded RNA. *Adv. Virus. Res*. 15: 61–158.
Ramsey, G. B. 1924. *Sclerotinia intermedia* n sp. a cause of decay of salsify and carrots. *Phytopathology* 14: 323–327.
Rashid, K. Y. 2001. *Sclerotinia* stem infection in flax in western Canada. In: *Proceedings of Sclerotinia 2001, the XI International Sclerotinia Workshop* (C. S. Young and K. J. D. Hughes, eds.). York 8–12 July 2001, Central Science Laboratory, York, England, pp. 103–104.
Rashid, K. Y. and Dedio, W. 1992. Differences in the reaction of sunflower hybrids to *Sclerotinia* wilt. *Can. J. Plant Sci*. 72: 925–930.
Rashid, K. Y. and Swanson, J. 2001. Seed treatment for the control of *Sclerotinia* basel stalk rot/ wilt in sunflower. In: *Proceedings of Sclerotinia 2001, the XI International Sclerotinia Workshop* (C. S. Young and K. J. D. Hughes, eds.). York 8–12 July 2001, Central Science Laboratory, York, England, pp. 113–114.
Raynal, G. 1990. Kinetics of the ascospore production of *Sclerotinia trifoliorum* Erikss. In: *Growth chamber and under natural climatic conditions*: *Practical and epidemiological incidence. J. Agronomie* 10: 561–572.
Raynal, G., Gayraud, P., Mousset-Declas, C. and Serpeille, A. 1991. Possible way to controlling *Sclerotinia* rot in seed clover. *Fourrages* 127: 335–344.
Reeleder, R. D. 2004. The use of yeast for biological control of plant pathogen *Sclerotinia sclerotiorum. Biocontrol* 49: 583–594.
Regente, M. C., Oliva, C. R., Feldman, M. L., Castagnaro, A. P. and Delaconal, L. 1997. A sunflower leaf antifungal peptide active against *Sclerotinia sclerotiorum. Physiol. Plantarum* 100: 178–182.
Reglinski, T., Poole, P. R., Whitaker, G. and Hoyte, S. M. 1997. Induced resistance against *Sclerotinia sclerotiorum* in kiwi fruit leaves. *Plant Pathol*. 46: 716–721.
Regnault, Y. and Pierre, J. G. 1984. Control of *Sclerotinia sclerotiorum* (Lib.) de Bary on oilseed rape in France. *Aspects Appl. Biol*. 6: 355–360.
Reichard, S. L., Sulc, R. M. and Rhodes, L. H. 1997. Growth and reproduction of *Sclerotinia trifoliorum* as influenced by herbicides. *Mycologia* 89: 82–88.
Renard, M. and Brun, H. 1982. Screening for resistance to *Phoma lingam. SID d'Amelioration des Plantes (INRA) Le Rheu*, France, pp. 137–147.
Reyes, A. A. 1988. Suppression of *Sclerotinia sclerotiorum* and watery soft of celery by controlled atmosphere storage. *Plant Dis*. 72: 790–792.
Reymond, P., Deleage, G., Rascle, C. and Fevre, M. 1994. Cloning and sequence analysis of a polygalacturonase-encoding gene from the phytopathogenic fungus *Sclerotinia sclerotiorum. Gene* 146: 233–237.

Rho, S. P., Bang, J. K., Kim, S. K., Lee, J. I., Park, R. K. and Kwon, B. S. 1986. A new good quality and highly yielding rape variety "Hallayuchae." *Res. Reptr. RDA. Crops* 28: 208–211.

Rhodes, L. H. and Myers, D. K. 1988. Potential for control of *Sclerotinia* crown rot of alfalfa with fungicides. In: *Proceedings of 31st North American Alfalfa Improvement Conference* (J. B. Moutray and J. H. Elgin Jr., eds.). NAAIC, Beltsville, MD, 19–23 June, 1988, p. 71.

Rhodes, L. H., Myers, D. K. and van Kouren, R. W.1992. A biological disease forecasting system for fungicidal control of *Sclerotinia* crown and stem rot. In: *Proceedings of 31st North American Alfalfa Improvement Conference*, June 14–18.

Riddle, G. E., Burpee, L. L. and Boland, G. J. 1991. Virulence of *Sclerotinia sclerotiorum* and *S. minor* on dandelion (*Taraxacum officinale* L.). *Weed Sci*. 39: 109–118.

Ridgway, H.J., Rabeendran, N., Eade, K. and Stewart, A. 2001. Application timing of *Coniothyrium minitans* A69 influences bio-control of *Sclerotinia minor* in lettuce. *Proc. Conf. Palmerston North, New Zealand, N.Z. Plant Prot*. 54: 14–16.

Rio, L. E. del, Martinson, C. A., Yang, X. B., Rio, L. del, Nelson, B. D. (ed.) and Gulya, T. J. 1998. Control of *Sclerotinia* stem rot of soybeans with *Sporidesmium sclerotivorum*. In: *Proceedings of 1998 International Sclerotinia Workshop*. Fargo, ND, 9–12 September, 1998, pp. 64–65.

Rio, L. E. del, Martinson, C. A. and Yang, X. B. 2002. Biological control of *Sclerotinia* stem rot of soybean with *Sporidesmium sclerotivorum. Plant Dis*. 86: 999–1004.

Rio, L. E. del, Bradley, C. A. and Johnson, B. L. 2005. First report of white mold caused by *Sclerotinia sclerotiorum* on Echium (*Echium vulgare*). *Plant Dis*. 89: 684.

Riou, C., Freyssinet, G. and Fevre, M. 1991. Production of cell wall degrading enzymes by the phytopathogenic fungus *Sclerotinia sclerotiorum. Appl. Environ. Microbiol*. 57: 1478–1484.

Riou, C., Freyssinet, G. and Fevre, M. 1992. Purification and characterization of extra cellular pectinolytic enzymes produced by *Sclerotinia sclerotiorum. Appl. Environ. Microbiol*. 58: 578–583.

Rivera, M. C., Wright, E. R., Goldring, L. V., Perez, B. A. and Barreto, D. 2004. First report of dollar spot caused by *Sclerotinia homoeocarpa* on *Agrostis stolonifera* in Argentina. *Plant Dis*. 88: 1384.

Rodriguez, M. A., Venedikian, N., Bazzalo, M. E. and Godeas, A. 2004. Histopathology of *Sclerotinia sclerotiorum* attack on flower parts of *Helianthus annuus* heads in tolerant and susceptible varieties. *Mycopathologia* 157: 291–302.

Rodriguez, M. A., Cabrera, G. and Godeas, A. 2006. Cyclosporine A from a nonpathogenic *Fusarium oxysporum* suppressing *Sclerotinia sclerotiorum. J. Appl. Microbiol*. 100: 575–586.

Rogozheva, M. F. and Kochenkova, K. G. 1982. White and grey rots of sunflowers. *Zasehcita Rastenil* 5: 20.

Rolland, S., Jobic, C., Fevre, M. and Bruel, C. 2003. *Agrobacterium* mediated transformation of *Botrytis cinerea* simple purification of monokaryotic transformants and rapid conidia based identification of the transfer DNA host genomic DNA flanking sequences. *Curr. Genet*. 44: 164–171.

Rollins, J. A. 2003. The *Sclerotinia sclerotiorum pac1* gene is required for sclerotial development and virulence. *Mol. Plant Microbe Interact*. 16: 785–795.

Rollins, J. A. and Dickman, M. B. 1998. Increase in endogenous and exogenous cyclic AMP levels inhibits sclerotial development in *Sclerotinia sclerotiorum. Appl. Environ. Microbiol*. 64: 2539–2544.

Rollins, J. A. and Dickman, M. B. 2001. pH signaling in *Sclerotinia sclerotiorum* identification of a *pac C/ RIM 1* homolog. *Appl. Environ. Microbiol*. 67: 75–81.

Ronicke, S., Hahn, V., Vogler, A. and Friedt, W. 2005. Quantitative trait loci analysis of resistance to *Sclerotinia sclerotiorum* in sunflower. *Phytopathology* 95: 834–839.

Rossman, A. Y., Palm, M. E. and Spielman, L. J. 1987. *A Literature Guide for the Identification of Plant Pathogenic Fungi*. American Phytopathological Society, St. Paul, MN, pp. 202–203.

Rotem, J. and Palti, J. 1969. Irrigation and plant diseases. *Ann. Rev. Phytopathol*. 7: 267–288.

Rowe, D. E. 1990. *Sclerotinia* crown and stem rot on two clover species and alfalfa. *Prog. Rep. Clovers Spec. Purpose Legumes Res*. 23: 26.

Rowe, D. E. 1993. Oxalic acid effects in exudates of *Sclerotinia trifoliorum* and *S. sclerotiorum* and potential use in selections. *Crop Sci*. 33: 1146–1149.

Rowe, D. E. and Welty, R. E. 1984. Indirect selection for resistance to *Sclerotinia* crown and stem rot on alfalfa. *Can. J. Plant. Sci*. 64: 145–150.

Roy, A. K. and Saikia, U. N. 1976. White blight of mustard and its control. *Indian J. Agric. Sci*. 46: 274–277.

Rubatzky, V. E., Quiros, C. F. and Simon, P. W. 1999. *Carrots and Related Vegetable Umbelliferae*. CAB International, New York.

Ruddick, J. A. and Harwing, J. 1975. Prenatal effects caused by feeding sclerotia of *Sclerotinia sclerotiorum* to pregnant rats. *Bull. Environ. Contam. Toxicol*. 13: 524–526.

Russo, G. M., Dahlberg, K. R. and van Etten, J. L. 1982. Identification of a development specific protein in sclerotia of *Sclerotinia sclerotiorum. Exp. Mycol*. 6: 259–267.

Ryley, M. J., Kyei, N. A. and Tatnell, J. R. 2000. Evaluation of fungicides for the management of *Sclerotinia* blight of peanut. *Aust. J. Agric. Res*. 51: 917–924.

Saindon, G. and Huang, H. C. 1992. Solid seeded upright beans avoid white mold. *Annu. Rep. Bean Improv. Coop*. 35: 95–96.

Saindon, G., Huang, H. C., Kozub, G. C., Mundel, H. H. and Kemp, G. A. 1993. Incidence of white mold and yield of upright bean grown in different planting patterns. *J. Phytopathol*. 137: 118–124.

Saindon, G., Huang, H. C. and Kozub, G. C. 1995. White mold avoidance and agronomic attributes of upright common bean grown at multiple planting densities in narrow rows. *J. Am. Soc. Hort. Sci*. 120: 843–847.

Saindon, G., Mundel, H. H. and Huang, H. C. 1996. Registration of AC Skipper, navy bean. *Crop Sci*. 36: 207.

Saito, I. 1969. Effect of some nutritional conditions on the formation and germinability of sclerotia of *Sclerotinia sclerotiorum* (Lib.) de Bary. *Bull. Hokkaido Pref. Agric. Exp. Sta*. 19: 1–7.

Saito, I. 1973. Initiation and development of apothecial stipe primordia in sclerotia of *Sclerotinia sclerotiorum. Trans. Mycol. Soc., Japan*. 14: 343–351.

Saito, I. 1974a. Utilization of β-glucans in germinating sclerotia of *Sclerotinia sclerotiorum* (Lib.) de Bary. *Ann. Phytopathol. Soc*. [Japan] 40: 372–374.

Saito, I. 1974b. Ultrastructural aspects of the maturation of sclerotia of *Sclerotinia sclerotiorum* (Lib.) de Bary. *Trans. Mycol. Soc*. [Japan] 15: 384–400.

Saito, I. 1977. *Sclerotinia sclerotiorum* (Lib.) de Bary. Studies on the maturation and germination of sclerotia of *Sclerotinia sclerotiorum* (Lib.) de Bary, a causal fungus of bean stem rot. *Report Hokkaido Prefectural Agric. Exp. Stn. No. 26, March, 1977*. Hokkaido Central Agricultural Experiment Station, 069–13, Japan, 106 p.

Saito, I. 1997. *Sclerotinia nivalis* sp. nov. the pathogen of snow mold of herbaceous dicots in northern Japan. *Mycoscience* 38: 227–236.

Saito, I. and Tkachenko, O. B. 2003. Low temperature species of *Sclerotinia* causing plant diseases in winter. *Adv. Plant Dis. Managt*. 195–214.

Sala, C. A., Vazquez, A. N., Romano, A. B. de and Prubello, S. 1994. *Sclerotinia* mid-stalk rot of sunflower, effect on yield components and oil content. *Helia* 17: 41–44.

Sands, D. C., Ford, E. J. and Miller, R. V. 1990. Genetic manipulation of broad host range fungi for biological control of weeds. *Weed Technol*. 4: 471–474.

Sansford, C. 1995. Oilseed rape: development of stem rot (*Sclerotinia sclerotiorum*) and its effect on yield. In: *Proceedings of IX International Rapeseed Congress*, Vol. 2, Rapeseed, Today and Tomorrow, Cambridge, pp. 634–636.

Sandys-Winsch, D. C., Whipps, J. M., Fenlon, J. S. and Lynch, J. M. 1994. The validity of *in vitro* screening methods in the search for fungal antagonists of *Sclerotinia sclerotiorum* causing wilt of sunflower. *Biocont. Sci. Technol*. 4: 269–277.

Sanudo, S. B. 1975. Some aspects of the nematode *Aphelenchoides* sp. its effect on the development of various soil fungi. *Noticias Fitopatologicas* 4: 148–159.

Saur, R. 1983. Experiments with spore trials for timing the control of *Sclerotinia sclerotiorum* on winter rape. *Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz* 90: 225–231.

Sawada, K. 1919. Descriptive catalogue of the Fonnosan fungi. Part I. *Agric. Exp. Stat. Govt. Fonnosa. Bull*. 9: 695.

Saxena, V. C. and Rai, J. N. 1987. Survey of occurrence of white rot of crucifers caused by *Sclerotinia sclerotiorum* in Uttar Pradesh and Bihar. *Indian J. Mycol. Plant Pathol*. 17: 89–91.

Scheel, D., Perone, V. B., Larkin, R. L. and Kupel, R. E. 1963. The isolation and characterization of two phototoxic furanocoumarins (Psoralens) from diseased celery. *Biochemistry* 2: 1127–1131.

Scheibert-Bohm, F., Schlosser, E. and Schuster, W. 1981. Studies on the infection of rape by *Sclerotinia sclerotiorum. Angew. Botanik* 55: 37–47.

Schleier, S., Voigt, K. and Wostemeyer, J. 1997. RAPD-based molecular diagnosis of mixed fungal infection on oilseed rape (*Brassica napus*): Evidence for genus and species specific sequences in the fungal genomes. *J. Phytopathol*. 145: 81–87.

Schmidt, D. 1981. Observations on the persistence of red clover. *Revue Suisse Agric*. 13: 149–158 (Sw.).

Schnick, P. J., Stewart-Wade, S. M. and Boland, G. J. 2002. 2, 4-D and *Sclerotinia minor* to control common dandelion. *Weed Sci*. 50: 173–178.

Schwartz, H. F. 1977. Epidemiology of white mold disease (*Sclerotinia sclerotiorum*) = (*Whetzelinia sclerotiorum*) of dry edible beans (*Phaseolus vulgaris*) with emphasis on resistance and host architectural disease avoidance mechanism. Ph.D. thesis University of Nebraska, Lincoln, Nebraska, 145 pp.

Schwartz, H. F. and Steadman, J. R. 1978. Factors affecting sclerotium population of and apothecium production by *Sclerotinia sclerotiorum. Phytopathology* 68: 383–388.

Schwartz, H. F., Steadman, J. R. and Coyne, D. R. P. 1978. Influence of *Phaseolus vulgaris* blossoming characteristics and canopy structure upon relation in *Sclerotinia sclerotiorum. Phytopathology* 68: 465–470.

Schwartz, H. F., Casciano, D. H., Asenga, J. A. and Wood, D. R. 1987. Field measurement of white mold effects upon dry beans with genetic resistance on upright plant architecture. *Crop Sci*. 27: 699–702.

Scott-Craig, J. S., Panaccione, D., Cervone, F. and Walton, J. D. 1990. Endopoly-galacturonase is not required for pathogenicity of *Cochliobolus carbonum* on maize. *Plant Cell* 2: 1191–1200.

Scott, S. W. 1981a. Separation of *Sclerotinia* isolates collected from three herbage legume hosts. *Trans. Br. Mycol. Soc*. 76: 321–323.

Scott, S. W. 1981b. Contamination of seed of white clover (*Trifolium repens*) with mycelium of a *Sclerotinia* species. *Trans. Br. Mycol. Soc*. 77: 671–674.

Scott, S. W. 1981c. Serological relationship of three *Sclerotinia* species. *Trans. Br. Mycol. Soc*. 77: 674–676.

Scott, S. W. and Evans, D. R. 1984. Sclerotia of *Sclerotinia trifoliorum* in red clover seed. *Trans. Br. Mycol. Soc*. 82: 567–569.

Sedun, F. S. and Brown, J. F. 1987. Infection of sunflower leaves by ascospores of *Sclerotinia sclerotiorum. Ann. Appl. Biol*. 110: 275–285.

Sedun, F. S. and Brown, J. F. 1989. Comparison of three methods to assess resistance in sunflower to basal stem rot caused by *Sclerotinia sclerotiorum* and *S. minor. Plant Dis*. 73: 52–55.

Sedun, F. S., Seguin-Swartz, G. and Rakow, G. F. W. 1989. Genetic variation in reaction to *Sclerotinia sclerotiorum* in *Brassica* species. *Can. J. Plant Sci*. 69: 229–232.

Seiler, G. J. 1991. Registration of six inter-specific sunflower germplasm lines derived from wild perennial species. *Crop Sci*. 31: 1097–1098.

Sexton, A. C. and Howlett, B. J. 2004. Microsatalite markers reveal genetic differentiation among populations of *Sclerotinia sclerotiorum* from Australian fields. *Curr. Genet*. 46: 357–365.

Shakeel, M. A. and Mowat, D. J. 1992. The potential transmission of clover rot, *Sclerotinia trifoliorum*. Erikss. by slugs. *Grass Forage Sci*. 47: 199–202.

Sharifi-Tehrani, A. 1974. Effect of seed disinfection to control sunflower foot rot caused by *Sclerotinia sclerotiorum* (Lib.) de Bary. *Iran J. Plant Pathol*. 10: 45.

Sharma, C. L. and Kapoor, A. S. 1999a. *In vitro* evaluation of herbicides and plant extracts against *Sclerotinia sclerotiorum* causing white rot in pea. *Plant Dis. Res*. 14: 79–81.

Sharma, C. L. and Kapoor, A. S. 1999b. Evaluation of resistance of pea germplasm against *Sclerotinia sclerotiorum. Himachal J. Agric. Res*. 25: 90–92.

Sharma, P. and Sain, S. K. 2004. Induction of systemic resistance in tomato and cauliflower by *Trichoderma* spp. against stalk rot pathogen *Sclerotinia sclerotiorum* (Lib) de Bary. *J. Biol. Control* 18: 21–27.

Sharma, P., Zewain, Q. K., Bahadur, P. and Sain, S. K. 2005. Effect of soil solarization on sclerotial viability of *Sclerotinia sclerotiorum* infecting cauliflower (*Brassica oleracea* var. *botrytis* sub var. *cauliflora*). *Indian J. Agril. Sci*. 75: 90–94.

Sharma, R. C. and Gill, S. S. 1996. Cultural control of *Sclerotinia sclerotiorum* under irrigated rice farming system. *Seed Res*. 24: 171–173.

Sharma, R. C. and Sharma, S. L. 1984a. Studies on pectolytic enzymes associated with stalk rot of cauliflower caused by *Sclerotinia sclerotiorum* (Lib.) de Bary. *Indian J. Plant Pathol*. 2: 69–72.

Sharma, R. C. and Sharma, S. L. 1984b. Evaluation and economics of fungicidal spray against *Sclerotinia* rot of cauliflower seed crop. *Seed Res*. 12: 95–97.

Sharma, R. C. and Sharma, S. L. 1986a. Survival of *Sclerotinia sclerotiorum* and initial infection of cauliflower in Himachal Pradesh. *Indian J. Ecology* 13: 367–369.

Sharma, R. C. and Sharma, S. L. 1986b. Effect of soil amendment and artificial defoliation of infected leaves on *Sclerotinia* stalk rot of cauliflower. *Indian J. Plant Pathol*. 4: 126–128.

Sharma, S. 2004. Influence of soil and water characteristics on white rot incidence in Indian mustard. *J. Mycol. Plant Pathol*. 34: 431–432.

Sharma, S., Yadav, J. L. and Sharma, G. R. 2001. Effect of various agronomic practices on the incidence of white rot of Indian mustard caused by *Sclerotinia sclerotiorum. J. Mycol. Plant Pathol*. 31: 83–84.

Sharma, S. L., Sharma, R. C. and Sharma, I. 1983. Cellulase activity of *Sclerotinia sclerotiorum* causing stalk rot of cauliflower. *Indian J. Mycol. Plant Pathol*. 13: 286–289.

Sharma, S. R., Kapoor, K. S. and Gill, H. S. 1995. Screening against *Sclerotinia* rot (*Sclerotinia sclerotiorum*), downy mildew (*Peronospora parasitica*) and black rot (*Xanthomonas compestris*) in cauliflower (*Brassica oleracea* var. *botrytis* sub. var. *cauliflora*). *Indian J. Agric. Sci*. 65: 916–918.

Shen, W. Z. 1992. Control of *Sclerotinia* rot of rape (*Sclerotinia sclerotiorum* de Bary) with carbendazim L S. *Plant Prot*. 18: 50.

Sherf, A. F. and Macnab, A. A. 1986. *Vegetable Diseases and Their Control*. Vol. II, Wiley, New York, 728 p.

Shi, Jun Ling, Li, Yin Qian Hua, Li, Du Guo Cheng and Chen, Jian. 2004. Pre-germinated conidia of *Coniothyrium minitans* enhances the foliar biological control of *Sclerotinia sclerotiorum. Biotech. Lett*. 26: 1649–1652.

Shi, Zhi Qi, Zhou, Ming Guo, Ye, Zhong Yin, Shi, Jian Rong, Chen, Huai Gu and Wang, Yu Zhong. 2000. Resistance monitoring of *Sclerotinia sclerotiorum* to carbendazim. *Jiangsu J. Agril. Sci*. 16: 226–229.

Shieh, M. T., Brown, R. L., Whitehead, M. P., Cary, J. W., Cotty, P. J., Cleveland, T. E. and Dean, R. A. 1997. Molecular genetic evidence for the involvement of a specific polygalacturonase P2c in the invasion and spread of *Aspergillus flavus* in cotton bolls. *Appl. Environ. Microbiol*. 63: 3548–3552.

Shindrova, P., Encheva, V. and Pechev, E. 1990. The effect of presowing seed treatment, seeding density and genotype of sunflower on attacks of the most widespread diseases. *Rasteniew dni-Nauki* 27: 49–55.

Shivpuri, A., Sharma, O. P. and Jhamaria, S. L. 1997. Fungitoxic properties of plant extracts against pathogenic fungi. *J. Mycol Plant Pathol*. 27: 29–31.

Shivpuri, A., Sharma, K. B. and Chhipa, H. P. 2000. Some studies on the stem rot (*Sclerotinia sclerotiorum*) disease of rapeseed-mustard in Rajasthan. *J. Mycol. Plant Pathol*. 30: 268.

Shivpuri, A., Bhargava, A. K. and Chhipa, H. P. 2001. *Sclerotinia sclerotiorum* – a new threat to mustard cultivation in Rajasthan, India. In: *Proceedings of Sclerotinia 2001, the XI*

International Sclerotinia Workshop (C. S. Young and K. J. D. Hughes, eds.). York 8–12, July, 2001, Central Science Laboratory, York, England, pp. 177–178.

Shopov, T. 1976. Contribution to the method of inoculation of sunflower by *Sclerotinia sclerotiorum*. (Lib.) Mass. *Rev. Plant Pathol*. 55: 1398 (Abstr.).

Shukla, A. K. 2005a. Estimation of yield losses to Indian mustard (*Brassica juncea*) due to *Sclerotinia* stem rot. *J. Phytol. Res*. 18: 267–268.

Shukla, A. K. 2005b. *Sclerotinia* rot - its prevalence in Indian mustard at different levels of nitrogen. *Indian Phytopath*. 58: 493–494.

Sigvald, R. 1985. Plant Protection year 1984. Agriculture. *Voxtskyddsnotiser* 49: 2–5.

Singh, D. 1991a. Development of white rot of pea (*Sclerotinia sclerotiorum*) in relation to meteorological factors. *Indian J. Mycol. Plant Pathol*. 21: 192–195.

Singh, D. 1991b. Biocontrol of *Sclerotinia sclerotiorum* (Lib.) de Bary by *Trichoderma harzianum. Trop. Pest Manage*. 37: 374–378.

Singh, D. 1995. Effect of sowing date on white rot (*Sclerotinia sclerotiorum*) development in pea (*Pisum sativum*). *Indian J. Agril. Sci*. 65: 621–623.

Singh, D. and Kapoor, A. S. 1996. Effects of fungicides on various growth stages of *Sclerotinia sclerotiorum. Indian J. Mycol. Plant Pathol*. 26: 185–189.

Singh, D. P. and Singh, R. B. 1984a. Effect of date of sowing on the occurrence of *Sclerotinia* stem rot and wilt of gram (*Cicer arietinum* L). *Phytopathol. Z*. 109: 254–260.

Singh, D. P. and Singh, R. B. 1984b. Differential response of host and non-host substrate on germination of ascospores of *Sclerotinia sclerotiorum. Phytopathol. Z*. 110: 277–280.

Singh, D. P., Ram, D. and Tewari, J. P. 1990. Induction of resistance in chickpea (*Cicer arietinum*) by *Aegle marmelos* leaves against *Sclerotinia sclerotiorum. Zeitschrift fur Pflanzenkrankheiten und Pflanzen schutz*. 97: 439–443.

Singh, H. and Singh, H. 1993. Sclerotia of *Sclerotinia trifoliorum* on seed as source of primary infection of stem rot of berseem. *Indian Phytopath*. 46: 93–94.

Singh, H. and Singh, H. 1995. Cultural control of stem rot of berseem caused by *Sclerotinia trifoliorum* Erikss. *Plant Dis. Res*. 10: 28–32.

Singh, H. B., Kalra, A., Patra, N. K., Singh, A., Tripathi, A. and Johri, J. K. 2000. *Sclerotinia* rot and blight of *Abelmoschus moschatus*: a new disease. *Indian Perfumer* 44: 3–5.

Singh, H., Kalda, T. S. and Singh, H. 1995a. Screening of cauliflower germplasm against *Sclerotinia* rot. *Indian J. Genet. Plant Breed*. 55: 98–102.

Singh, K. P., Shahi, D. K. and Singh, U. P. 1995b. Carpogenic germination of sclerotia of *Sclerotinia sclerotiorum* in some soil samples differing in ion exchange and soil nutrient properties. *Trop. Sci*. 35: 354–358.

Singh, P. and Singh, H. B. 2003. A new disease of opium poppy (*Papaver somniferum* L.) caused by *Sclerotinia sclerotiorum* (Lib.) de Bary. *J. Mycol. Plant Pathol*. 33: 152–154.

Singh, R. and Tripathi, N. N. 1995. Effect of date of sowing and stage of crop growth on severity of *Sclerotinia* rot of sunflower (*Helianthus annuus* L) *Haryana Agric. Univ. J. Res*. 25: 131–132.

Singh, R. and Tripathi, N. N. 1996a. Influence of flooding frequency and sclerotial position on the management of stem rot of sunflower. *Agric. Sci. Digest*. 16: 75–77.

Singh, R. and Tripathi, N. N. 1996b. Management of *Sclerotinia* rot of sunflower by neemguard. *Agric. Sci. Digest*. 16: 15–16.

Singh, R. and Tripathi, N. N. 1996c. Effect of soil type on apothecial production of *Sclerotinia sclerotiorum* (Lib) de Bary. *Indian J. Agric. Res*. 30: 191–196.

Singh, R. and Tripathi, N. N. 1997. Management of *Sclerotinia* rot of sunflower by integration of cultural, chemical and biological methods. *J. Mycol. Plant Pathol*. 27: 67–70.

Singh, R. and Tripathi, N. N. 1998. Weather factors associated with production and release of ascospores of *Sclerotinia sclerotiorum. J. Mycol. Plant Pathol*. 28: 227–230.

Singh, R. B. and Singh, D. P. 1985. A modified method for collecting ascospores of *Sclerotinia sclerotiorum. Int. Chickpea Newslett*. 13: 21–22.

Singh, R. S. 1987. *Disease of Vegetable Crops*. Oxford/IBH Publishing, New Delhi, 362 p.

Singh, S. 2001. Management of *Sclerotinia* rot in Indian mustard–An integrated approach. In: *Proceedings of Sclerotinia 2001, the XI International Sclerotinia Workshop* (C. S. Young and

K. J. D. Hughes, eds.). York 8–12, July, 2001, Central Science Laboratory, York, England, pp. 139–140.

Singh, S. B. and Singh, R. 2001. A new *Sclerotinia* stem rot of bishop weed. *Indian Phytopath*. 54: 140.

Singh, S. B., Singh, A. K. and Singh, A. K. 2003. *In vitro* evaluation of fungitoxicants on developmental stages of *Sclerotinia sclerotiorum. Ann. Plant Prot. Sci*. 11: 388–390.

Singh, U. P., Sarma, B. K., Singh, D. P., Maurya, S., Mishra, P. K. and Singh, H. B. 2004. Effects of exudates depletion on sclerotial development of *Sclerotinia sclerotiorum* and of oxalic acid on the synthesis of phenolic acids in eggplant (*Solanum melongena* L.). *Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz* 111: 371–379.

Singh, V. P. and Singh, R. B. 1983. The effect of soil texture, soil moisture, soil mixture and depth of soil on carpogenic germination of *Sclerotinia sclerotiorum. J. Plant Dis. Prot*. 90: 662–669.

Singh, V. P., Singh, R. B. and Gupta, S. 1985. Effect of temperature on sclerotial germination, growth and sclerotial formation in *Sclerotinia sclerotiorum. J. Plant Dis. Prot*. 92: 281–287.

Sinha, J. M. and Singh, A. P. 1991. New host for *Oidiopsis taurica* and *Whetzelinia sclerotiorum. Indian Phytopath*. 44: 558.

Sinigaglia, C., Patricio, F. R. A., Ghini, R., Malavolta, V. M. A., Tessarioli, J. and Freitas, S. dos. 2001. *Sclerotinia minor*, *Rhizoctonia solani* and weed infestation control in lettuce by soil solarization alone or integrated with chemical control. *Summa Phytopathologica* 27: 229–235.

Sirjusingh, C. and Kohn, L. M. 2001. Characterization of micro-satellites in the fungal plant pathogen *Sclerotinia sclerotiorum. Mol. Ecol. Notes* 1: 267–269.

Sitepu, D. and Wallace, H. R. 1984. Biological control of *Sclerotinia sclerotiorum* in lettuce by *Fusarium lateritium. Aust. J. Exp. Agri. Anim. Husb*. 24: 272–276.

Siu, R. G. H. 1951. *Microbial Decomposition of Cellulose*. Rheinhold Publishing,, New York.

Skoric, D. and Rajcan, I. 1992. Breeding for *Sclerotinia* tolerance in sunflower. In: *Proceedings of 13th International Sunflower Conference*, Vol. 1, Pisa, Italy, 7–11 September, pp. 1257–1262.

Skoric, D., Atlagic, J., Marinkovic, R., Dozet, B. and Mihaljevic, M. 1995. *FAO working group: evaluaiton of wild Helianthus species. Progress Report, 1994. Bucharest, D Romania*, pp. 11–25.

Smith, A. M. 1972. Biological control of fungal sclerotia in soil. *Soil Biol. Biochem*. 4: 131–134.

Smith, B. A. and Boland, G. J. 1989. A reliable method for the production and maintenance of germinated sclerotia of *Sclerotinia sclerotiorum. Can. J. Plant Pathol*. 11: 45–48.

Smith, F. D., Phipps, P. M. and Stipes, R. J. 1991a. Use of pinolene or other spray adjuvant with iprodione for improved control of *Sclerotinia* blight of peanut. *Peanut Sci*. 18: 97–101.

Smith, F. D., Phipps, P. M. and Stipes, R. J. 1991b. Agar plate, soil plate and field evaluation of fluazinam and other fungicides for control of *Sclerotinia minor* on peanut. *Plant Dis*. 75: 1138–1143.

Smith, J. D. 1955. Fungi and turf diseases. *J. Sports Turf. Res. Inst*. 9: 35–59.

Smith, O. D., Aguisre, S. M., Boswell, T. E., Grichar, W. J., Melouk, H. A. and Simpson, C. F. 1990. Registration of TxAG-4 and TxAG-5 peanut germplasm. *Crop Sci*. 30: 429.

Smith, R. E. 1929. Life history of *Sclerotinia sclerotiorum* (Lib.) Mass in connection with green rot of apricots. *Phytopathology* 19: 1136–1137.

Smith, R. E. 1900. *Botrytis* and *Sclerotinia*: their relation to certain plant diseases and to each other. *Bot. Gaz.* 29: 369–407.

Smith, R. J., Jr. 1991. Integration of biological control agents with chemical pesticides. In: *Microbial Control of Weeds* (D. O. Tebeest, ed.). Chapman & Hall, New York, pp. 189–208.

Smith, V. L., Punja, Z. K. and Jenkins, S. F. 1986. A histological study of infection of host tissue by *Sclerotinia rolfsii. Phytopathology* 76: 755–759.

Song, J. H. and Koh, Y. J. 1999. *Sclerotinia* twig blight on trees and cottony rot on fruits of satsuma mandarin caused by *Sclerotinia sclerotiorum. Plant Pathol. J*. 15: 236–241.

Spitsyn, V. P. and Kochenkova, K. G. 1978. Pathogenic properties of sclerotia of white rot in relation to the length of their survival and depth in the soil. *Rev. Plant Pathol*. 57: 243 (Abstr.).

Starr, G. H., Walters, H. J. and Bridgmon, G. H. 1953. White mould (*Sclerotinia*) of beans. *Wyoming Agric. Exp. Sta. Tech. Bull*. 322: 11.

Starzycka, E., Starzycki, M., Cichy, H. and Mikolajczyk, K. 1998. Examination of resistance of winter oilseed rape to *Sclerotinia sclerotiorum* (Lib.) de Bary. *Rosliny Oleiste* 19: 493–500.

Starzycka, E., Starzycki, M., Pszczola, J. and Mikolajczyk, K. 2000. Stem rot resistance degree of winter oilseed rape strains (*Brassica napus* L.) in 1999 and investigations on *Sclerotinia sclerotiorum* (Lib.) de Bary pathotypes. *Rosliny Oleiste*. 21: 391–398.

Starzycka, E., Starzycki, M. and Pszczola, J. 2001. Obtaining of pure *Sclerotinia sclerotiorum* (Lib.) de Bary isolates from contaminated sclerotia. *Rosliny Oleiste* 22: 455–461.

Starzycka, E., Starzycki, M., Cichy, H., Cicha, A., Budzianowski, G. and Szachnowska, H. 2004. Resistance of some winter rapeseed (*Brassica napus* L.) cultivars to *Sclerotinia sclerotiorum* (Lib.) de Bary infection. *Rosliny Oleiste*. 25: 645–654.

Steadman, J. R. 1975. Nature and epidemiological significance of infection of bean seed by *Whetzelinia sclerotiorum. Phytopathology* 65: 1323–1324.

Steadman, J. R. 1979. Control of plant diseases caused by *Sclerotinia* species. *Phytopathology* 69: 904–907.

Steadman, J. R. 1983. White mold - A serious yield limiting disease of bean. *Plant Dis*. 67: 346–350.

Steadman, J. R. and Cook, G. B. 1974. A simple method for collecting ascospores of *Whetzelinia sclerotiorum. Plant Dis. Reptr*. 58: 190.

Steadman, J. R. and Nickerson, K. W. 1975. Differential inhibition of sclerotial germination in *Whetzelinia sclerotiorum. Mycopathologia* 57: 165–170.

Steadman, J. R., Coyne, D. P. and Cook, G. E. 1973. Reduction of severity of white mold disease on great Northern bean by wider row spacing and determinate plant growth habit. *Plant Dis. Reptr*. 57: 1070–1071.

Steadman, J. R., Coyne, D. P. and Schwartz, H. F. 1974. Field reaction of beans to severe white mold infection. *Annu. Rep. Bean Improv. Coop*. 17: 84–85.

Steadman, J. R., Maier, L. R., Schwartz, H. F. and Kerr, B. D. 1975. Pollution of surface irrigation water by plant pathogenic organisms. *Water Resource Bull*. 11: 796–804.

Steadman, J. R., Jung, G., Adams, M. S., Powers, K., Higgins, B., Nelson, B. D. (ed.) and Gulya, T. J. 1998. Random amplified polymorphic DNA (RAPD) distinguishes three species of *Sclerotinia* but not pathogenic variability in *S. sclerotiorum* isolates from diverse host and geographic origin. In: *Proceedings of 1998 International Sclerotinia Workshop*, Fargo, ND, 9–12 September, 1998, pp. 10–13.

Stelfox, D., WilIams, J. R, Soehngen, U. and Topping, R. C. 1978. Transport of *Sclerotinia sclerotiorum* ascospores by rapeseed pollens in Alberta. *Plant Dis. Reptr*. 62: 576–579.

Stevens, F. L. and Hall, J. G. 1911. A serious lettuce disease (sclerotiniose) and a method of control. *N.C. Agric Exp. Stn. Tech. Bull.* 8: 85–145.

Stewart, A., Eade, K., Rabeendran, N., Ridgway, H. and Elad, Y. (ed.), Freeman, S. (ed.) and Monte, E. 2000. Mechanisms of biological control of *Sclerotinia* lettuce drop by *Coniothyrium minitans*-more than just sclerotial parasitism. IOBC-WPRS Working Group "*Biological Control of Fungal and Bacterial Plant Pathogens*". In: *Proceedings of Sixth Meeting, Biocontrol Agents: Mode of Action and Interaction with Other Means of Control*, Sevilla, Spain, November 30–December 3, 2000. *Bull. OILB-SROP* 24: 1–4.

Stiers, D. L., Fellman, J. K. and Tourneau, D. L. 1980. The effect of triadimefon on growth, sclerotial formation and ultrastructure of *Sclerotinia sclerotiorum. Environ. Exp. Bot*. 20: 81–89.

Stone, G. E. and Smith, R. E. 1900. The rotting of greenhouse lettuce. *Mass. Agric. Coll. Stn. Bull.* 69: 40.

Stone, H. E. and Armentrout, V. N. 1985. Production of oxalic acid by *Sclerotinia cepivorum* during infection of onion. *Mycologia* 77: 526–530.

Subbarao, K. V. 1996. Epidemiology and control of lettuce drop caused by *Sclerotinia minor. Ann. Lettuce Res. Rep*. Crop year 1995. California Iceberg Lettuce Advisory Board, Salinas.

Subbarao, K. V. 2002. Cottony rot/Pink rot. In: *Compendium of Umbelleferous Crop Diseases* (R. M. Davis and R. N. Raid, eds.). American Phytopathological Society, St. Paul, MN, pp. 29–30.

Subbarao, K. V., Hubbard, J. E. and Schulbach, K. F. 1994. Effect of irrigation methods on disease management in lettuce. *Phytopathology* 84: 1103–1104 (Abstr.).

Subbarao, K. V., Koike, S. T. and Hubbard, J. C. 1996. Effects of deep ploughing on the distribution and density of *Sclerotinia minor* sclerotia and lettuce drop incidence. *Plant Dis*. 80: 28–33.

Subbarao, K. V., Hubbard, J. E. and Schulbach, K. F. 1997. Comparison of lettuce disease and yield under subsurface drop and furrow irrigation. *Phytopathology* 87: 877–883.

Subbarao, K. V., Hubbard, J. C., Koike, S. T., Nelson, B. D. (ed.) and Gulya, T. J. 1998. Dynamics of *Sclerotinia minor* sclerotia under a lettuce-broccoli rotation system. In: *Proceedings of 1998 International Sclerotinia Workshop*. Fargo, ND, 9–12 September, pp. 26–27.

Sugha, S. K. 1999. Efficacy of carbendazim granules against *Sclerotinia sclerotiorum* of pea (*Pisum sativum*), cabbage (*Brassica oleracea* var. *capitata*) and cauliflower (*Brassica oleracea* var. *botrytis*). *Indian J. Agril. Sci*. 69: 302–303.

Sugha, S. K. 2001. Management of white rot of pea by fungicides. *J. Mycol. Plant Pathol*. 31: 335–338.

Sulc, R. M. and Rhodes, L. H. 1997. Planting date, fungicide and cultivar effects of *Sclerotinia* crown and stem rot severity in alfalfa. *Plant Dis*. 81: 13–17.

Sun, Jun Ming, Irzykowski, W., Jedryczka, M. and Han, Fen Xia 2005. Analysis of the genetic structure of *Sclerotinia sclerotiorum* (Lib.) de Bary populations from different regions and host plants by random amplified polymorphic DNA markers. *J. Integr. Plant Biol*. 47: 385–395.

Sun, P. and Yang, X. B. 2000. Light, temperature, and moisture effects on apothecium production of *Sclerotinia sclerotiorum. Plant Dis*. 84: 1287–1293.

Suneja, S., Lakshminarayana, K. and Gupta, P. P. 1994. Role of *Azotobacter chroococcum* sidrophores in control of bacterial rot and *Sclerotinia* rot of mustard. *Indian J. Mycol. Plant Pathol*. 24: 202–205.

Sutton, D. C. and Deverall, B. J. 1983. Studies on infection of bean (*Phaseolus vulgaris*) and soybean (*Glycine max*.) by ascospores of *Sclerotinia sclerotiorum. Plant Pathol*. 32: 251–261.

Sutton, J. C. and Peng, G. 1993. Manipulation and vectoring of biocontrol organisms to manage foliage and fruit diseases in cropping systems. *Ann. Rev. Phytopathol*. 31: 473–493.

Suzui, T. and Kobayashi, T. 1972a. Dispersal of ascospores of *Sclerotinia sclerotiorum* (Lib.) de Bary on kidney bean plants. Part 1. Dispersal of ascospores from a point source of apothecia. *Hokkaido Nat. Agric. Exp. Stn. Bull*. 101: 137–151.

Suzui, T. and Kobayashi, T. 1972b. Dispersal of ascospores of *Sclerotinia sclerotiorum* (Lib.) de Bary on kidney bean plants. Part 2. Dispersal of ascospores in the Tokachi District Hokkaido. *Hokkaido Nat. Agric. Exp. Stn. Res. Bull*. 102: 61–68.

Suzui, T. and Kobayashi, T. 1972c. Dispersal of ascospores of *Sclerotinia sclerotiorum* (Lib.) de Bary, on kidney bean plants. Part 3. Number of ascospores and environmental factors after infection. *Hokkaido Nat. Agric. Exp. Stn. Res. Bull*. 102: 69–82.

Suzzi, G., Romano, P., Panti, L. and Montuschi, C. 1995. National wine yeasts as biochemical agents. *J. Appl. Bacteriol*. 78: 304–308.

Swaminathan, J., McLean, K. L., Pay, J. M. and Stewart, A. 1999. Soil solarization: a cultural practice to reduce viability of sclerotia of *Sclerotinia sclerotiorum* in New Zealand soils. *N. Z. J. Crop Hortic. Sci*. 27: 331–335.

Tahvonen, R. 1985. The prevention of *Botrytis cinerea* and *Sclerotinia sclerotiorum* on carrots during storage by spraying the tops with fungicide before harvesting. *Ann. Agric. Fenn*. 24: 89–95.

Takeuchi, J. and Horie, H. 1996. Occurrence of *Sclerotinia* rot of *Brassica campestris* (Chinensis Group), *Angelica keiskei*, beard-tongue [Penstemon], bladder campion [*Silene vulgaris*] and verbena [*V. officinalis*] caused by *Sclerotinia sclerotiorum. Proc. Kanto Tosan Plant Prot. Soc*. 43: 67–70.

Takeuchi, J. and Horie, H. 1999. First occurrence of *Sclerotinia* rot in Aster and strawflower in Japan. *Ann. Report Kanto Tosan Plant Prot. Soc*. 46: 57–59.

Tanas, T. 2004. The results of examination safeguard cucumbers in plastic houses from attack by *Sclerotinia sclerotiorum* (Lib.) de Bary. *Glasnik Zastite Bilja* 27: 63–69.

Tapke, V. F. 1946. A rapid method for isolating single ascospore from apothecia. *Phytopathology* 36: 167–168.

Tarabeih, A. M. and Abou-EI-Fadl, L. A. 1979. Effect of *Sclerotinia sclerotiorum* on the volatile oil content of some medicinal plants. *Acta. Phytopathologia Academiae Scientiarum Hungaricae* 14: 31–35.

Tariq, V. N. 1984. *Sclerotinia* disease of plants: characterization of isolates and host pathogen interaction. Ph.D. thesis. University of Kent, Canterbury.

Tariq, V. N. and Jeffries, P. 1984. Appressorium formation by *Sclerotinia sclerotiorum: s*canning electron microscopy. *Tran. Br. Mycol. Soc*. 82: 645–651.

Tariq, V. N. and Jeffries, P. 1985. Changes occurring in chloroplast of *Phaseolus* following infection by *Sclerotinia:* a cytochemical study. *J. Cell Sci*. 75: 195–205.

Tariq, V. N. and Jeffries, P. 1986. Ultrastructure penetration of *Phaseolus* spp. by *Sclerotinia sclerotiorum. Can. J. Bot*. 64: 2909–2915.

Tariq, V. N. and Jeffries, P. 1987. Cytochemical localization of lipolytic enzyme activity during penetration of host tissues by *Sclerotinia sclerotiorum. Physiol. Mol. Plant Pathol*. 30: 77–91.

Tariq, V. N., Gutteridge, C. S. and Jeffries, P. 1985. Comparative studies of cultural and biochemical characteristics used for distinguishing species within *Sclerotinia. Trans. Br. Mycol. Soc*. 84: 381–397.

Taubenhaus, J.J. and Ezekiel, W.N. 1931. A *Sclerotinia* limb blight of figs. *Phytopathology* 21: 1195–1197.

TeBeest, D. O. 1996. Biological control of weeds with plant pathogens and microbial pesticides. In: *Advances in Agronomy* (D. L. Sparks ed.). Vol. 56, Academic, San Diego, CA, pp. 115–137.

TeBeest, D. O. and Templeton, G. E. 1985. Mycoherbicides: progress in the biological control of weeds. *Plant Dis*. 69: 6–10.

Templeton, G. E. 1982. Status of weed control with plant pathogens. In: *Biological Control of Weeds with Plant Pathogens* (R. Charudattan and H. L. Walker, eds.). Wiley, New York, pp. 29–44.

Teo, B. K. and Morrall, R. A. A. 1985a. Influence of matric potentials on carpogenic germination of sclerotia of *Sclerotinia sclerotiorum*. I. Development of an inclined box technique to observe apothecium production. *Can. J. Plant Pathol*. 7: 359–364.

Teo, B. K. and Morrall, R. A. A. 1985b. Influence of matric potentials on carpogenic germination of sclerotia of *Sclerotinia sclerotiorum*. II A Comparison of results obtained with different techniques. *Can. J. Plant Pathol*. 7: 365–369.

Teo, B. K., Verma, P. R. and Morrall, R. A. A. 1992. The effect of herbicides and mycoparasites at different moisture levels on carpogenic germination in *Sclerotinia sclerotiorum. Plant Soil* 139: 99–107.

Terui, M. and Harada, Y. 1966. Effect of low temperature treatment of sclerotia on apothecial production in *Sclerotinia sclerotiorum. Bull. Faclt. Agric. Hirosaki, Univ*. 12: 24–30.

Tetsuka, T. and Ishida, M. 2000. Evaluation of resistance to *Sclerotinia sclerotiorum* in rape cultivars (*Brassica napus* L.). *Reptr. Kyushu Br. Crop Sci. Soc*. [Japan] 66, 70–71.

Tewari, J. P. and Conn, K. H. 1992. Control of *Sclerotinia* on canola by calcium application. *Commission Project* 9101: 17.

Thaning, C. 2000. Ways of managing *Sclerotinia sclerotiorum* inoculum. *Acta Univ. Agric. Sueciae Agraria* 240, 1–124.

Thatcher, F. S. 1942. Further studies of osmotic and permeability relations in parasitism. *Can. J. Res*. (c) 20: 283–311.

Thomma, B. P. H. J. 2003. *Alternaria* spp. from general saprophyte to specific parasite. *Mol. Plant Pathol*. 4: 225–236.

Thomma, B. P. H. J., Penninckx, I. A. M. A., Broekaert, W. F. and Cammue, B. P. A. 2001. The complexity of disease signaling in *Arabidopsis. Curr. Opin. Immunol*. 13: 63–68.

Thomma, B. P. H. J., van Esse, H. P., Crous, P. W. and de Wit, P. J. G. M. 2005. *Cladosporium fulvum* (syn. *Passalora fulva*) a highly specialized plant pathogen as a model for functional studies on plant pathogenic *Mycosphaerellaceae. Mol. Plant Pathol*. 6: 379–393.

Thompson, A. H. and Westhuizen, C. A. 1979. *Sclerotinia sclerotiorum* (Lib.) de Bary, on soybean in South Africa. *Phytophytactica* 11: 145–148.

Thompson, J. R. and Kondra, Z. P. 1983. Techniques for artificially inoculating oilseed rape with *Sclerotinia sclerotiorum. Proceedings of Sixth International Rapeseed Conference*, 2: 185 (Abstr.).

Thompson, J. R., Thomas, P. M. and Evans, I. R. 1984. Efficacy of aerial application of benomyl and iprodione for the control of *Sclerotinia* stem rot of canola (rapeseed) in Central Alberta. *Can. J. Plant Pathol*. 6: 75–77.

Toal, E. S. and Jones, P. W. 1999. Induction of systemic resistance to *Sclerotinia sclerotiorum* by oxalic acid in oilseed rape. *Plant Pathol*. 48: 759–767.

Togawa, M. 2000. First occurrence of *Sclerotinia* rot (*Sclerotinia sclerotiorum*) in perilla (*Perilla frutescens*) and bupleurum (*Bupleurum rotundifolium*) in Japan. *Proc. Kansai Plant Prot. Soc*. 42: 77–78.

Tollenaar, H. and Beleiholder, H. 1972. Distribution of the mycelium of *Sclerotinia sclerotiorum* in sunflower seed. *Rev. Plant Pathol*. 31: 3487 (Abstr.).

Torrington, T. K., Morrall, R. A. A. and Gurgle, R. K. 1991. Use of petal infestation to forecast *Sclerotinia* stem rot of canola: evaluation of early bloom sampling 1985–90. *Can. J. Plant Pathol*. 13: 50–59.

Toth, I. K., Bell, K. S., Holeva, M. C. and Birch, P. R. J. 2003. Soft rot *Erwiniae* from genes to genomes. *Mol. Plant Pathol*. 4: 17–30.

Townsend, B. D. and Willetts, H. J. 1954. The development of sclerotia of certain fungi. *Trans. Br. Mycol. Soc*. 37: 213–221.

Trevethick, J. and Cooke, R. C. 1971. Effect of some metabolic inhibitors and sulfur containing amino acids on sclerotium formation in *Sclerotium rolfsii, S. delphini* and *Sclerotinia sclerotiorum. Trans. Br. Mycol. Soc*. 57: 340–341.

Trevethick, J. and Cooke, R. C. 1973. Non nutritional factors influencing sclerotia formation in some *Sclerotinia* and *Sclerotium* species. *Trans. Br. Mycol. Soc*. 60: 559–566.

Tribe, H. T. 1957. On the parasitism of *Sclerotinia trifoliorum* by *Coniothyrium minitans. Trans. Br. Mycol. Soc*. 40: 489–499.

Tripolka, M. G. 1977. Purification of sunflower seeds from sclerotia. *Zashehita Rastenii* 3: 31.

Tronsmo, A. 1989. *Trichoderma harzianum* used for biological control of storage rot of carrots. *Norwegian J. Agri. Sci*. 3: 157–161.

Trutmann, P. and Keane, P. J. 1990. *Trichoderma koningii* as a biological control agent for *Sclerotinia sclerotiorum* in Southern Australia. *Soil Biol. Biochem*. 22: 43–50.

Trutmann, P., Keane, P. J. and Merriman, P. R. 1980. Reduction of sclerotial inoculum of *Sclerotinia sclerotiorum* with *Coniothyrium minitans. Soil Biol. Biochem*. 12: 461–465.

Trutmann, P., Keane, P. J. and Merriman, P. R. 1982. Biological control of *Sclerotinia sclerotiorum* on aerial parts of plants by the hyperparasite *Coniothyrium minitans. Trans. Br. Mycol. Soc*. 78: 521–529.

Trutmann, P., Keane, P. J. and Merriman, P. R. 1983. Influence of environmental factors and sclerotial origin on parasitism of *Sclerotinia sclerotiorum* by *Coniothyrium minitans*. In: *Proceedings of 4th International Congress Plant Pathology*. University of Melbourne, Australia, pp. 221–223.

Tu, E. C. and Kimbrough, J. W. 1973. A rapid staining technique for *Rhizoctonia solani* and related fungi. *Mycologia* 65: 941–944.

Tu, J. C. 1980. *Gliocladium virens* - a destructive mycoparasite of *Sclerotinia sclerotiorum. Phytopathology* 70: 670–674.

Tu, J. C. 1984. Mycoparasitism by *Coniothyrium minitans* on *Sclerotinia sclerotiorum* and its effect on sclerotial germination. *Phytopath*. Z. 109: 261–268.

Tu, J. C. 1985. Tolerance of white bean (*Phaseolus vulgaris*) to white mould (*Sclerotinia sclerotiorum*) associated with tolerance to oxalic acid. *Physiol. Plant Pathol*. 26: 111–117.

Tu, J. C. 1988. The role of white mold infected white bean (*Phaseolus vulgaris* L) seeds in The dissemination of *Sclerotinia sclerotiorum* (Lib.) de Bary. *J. Phytopathol*. 121: 40–50.

Tu, J. C. 1989a. Oxalic acid induced cytological alterations differ in bean tolerance or susceptibility to white mould. *New Phytol*. 112: 519–525.

Tu, J. C. 1989b. Modes of primary infection caused by *Sclerotinia sclerotiorum* in navybean. *Microbios*. 57: 85–91.

Tu, J. C. 1989c. Management of white mold of white beans in Ontario. *Plant Dis*. 73: 281–285.

Tu, J. C. 1997. An integrated control of white mold (*Sclerotinia sclerotiorum*) of beans with emphasis on recent advances in biological control. *Bot. Bull. Academia Sinica* 38: 73–76.

Tu, J. C. and Beversdorf, W. D. 1982. Tolerance to white mold (*Sclerotinia sclerotiorum*) (Lib.) de Bary in Ex. Rico. 23, a cultivar of white bean (*Phaseolus vulgaris* L). *Can. J. Plant Sci*. 62: 65–69.

Turkington, T. K. and Morrall, R. A. A. 1993. Use of petal infestation to forecast *Sclerotinia* stem rot of canola- the influence of inoculum variation over the flowering period and canopy density. *Phytopathology* 83: 682–689.

Turkington, T. K., Morrall, R. A. A. and Gugel, R. K. 1991a. Use of petal infestation to forecast *Sclerotinia* stem rot of canola: evaluation of early bloom sampling 1985–90. *Can. J. Plant Pathol*. 13: 50–59.

Turkington, T. K., Morrall, R. A. A. and Rude, S. V. 1991b. Use of petal infestation to forecast *Sclerotinia* stem rot of canola: the impact of diurnal and weather related inoculum fluctuations. *Can. J. Plant Pathol*. 13: 347–355.

Turner, G. J. and Tribe, H. T. 1976. On *Coniothyrium minitans* and its parasitism on *Sclerotinia* species. *Trans. Br. Mycol. Soc*. 66: 97–105.

Turner, J., Young, C., Riding, A. and Gladders, P. 2002. *Sclerotinia* control in oilseed rape: progress with quantitative diagnosis and development of a web based forecasting scheme. *HGCA Project Report* 0S56: 76.

Twengstrom, E., Sigvald, R., Svensson, C. and Yuen, J. 1998a. Forecasting *Sclerotinia* stem rot in spring sown oilseed rape. *Crop Prot*. 17: 405–411.

Twengstrom, E., Kopmans, E., Sigvald, R. and Svensson, C. 1998b. Influence of different irrigation regimes on carpogenic germination of sclerotia of *Sclerotinia sclerotiorum. J. Phytopath*. 146: 487–493.

Uecker, F. A., Ayers, W. A. and Adams, P. B. 1978. A new hyphomycetes on sclerotia of *Sclerotinia sclerotiorum. Mycotaxon* 7: 275–282.

Uecker, F. A., Ayers, W. A. and Adams, P. B. 1980. *Teratosperma oligocladium,* a new hyphomycetes mycoparasite on sclerotia of *Sclerotinia sclerotiorum, S. trifoliorum* and *S. minor. Mycotaxon* 10: 421–427.

Upadhyaya, J. 1994. *Sclerotinia* collar-rot of *Ammi majus* Linn. *Prog. Hort*. 26: 77–78.

Upadhyay, R. S. and Jayaswal, R. K. 1992. *Pseudomonas cepacia* causes mycelial deformities and inhibition of conidia in phytopathogenic fungi. *Curr. Microbiol*. 24: 181–187.

Utkhede, R. S. and Rahe, J. E. 1979. Wet sieving floatation technique for isolation of sclerotia of *Sclerotium cepivorum* from muck soil. *Phytopathology* 69: 295–297.

Valleau, W. D., Fergus, E. N. and Henson, L. 1933. Resistance of red clover to *Sclerotinia trifoliorum* Eriss. and infection studies. *Ky. Agric. Exp. Stn. Bull*. 341: 113–131.

Van Alfen, N. K. 1982. Biology and potential for disease control of hypovirulence of *Endothia parasitica. Ann. Rev. Phytopathol*. 20: 349–362.

Van den Berg, L. and Yang, S. M. 1969. Effect of relative humidity on production of extra-cellular pectolytic enzymes by *Botrytis cinerea* and *Sclerotinia sclerotiorum. Can. J. Bot*. 47: 1007–1010.

Vaughan, D. and Jones, D. 1979. Effects of Dazomet on phenolase activity in sclerotia of *Sclerotinia sclerotiorum. J. Exp. Bot*. 30: 751–758.

Vear, F. and Guillaumin, J. J. 1977. Study of methods of inoculating sunflower with *Sclerotinia sclerotiorum* and their application to selection. *Annules de* L *Amelioration des Plantes* 27: 523–537.

Vega, R. R. and Le Tourneau, D. 1974. The effect of zinc on the growth and sclerotial formation of *Whetzelinia sclerotiorum. Mycologia* 66: 256–264.

Verkaaik, M. L., Hurrell, G. A., Bourdot, G. W. and Saville, D. J. 2004. Evaluation of *Sclerotinia sclerotiorum* for giant buttercup control in dairy pastures. New Zealand Plant Prot., *Proceedings of Conference, Hamilton*, Vol. 57, No. 4, New Zealand, 10–12 August, pp. 286–291 (Abstr.).

Vernuseu, I. and Iliescu, H. 1980. The control of mould fungi in sunflower by seed treatment. *Rev. Plant Pathol* 59: 1825 (Abstr.).

Viana, F. M. P., Kobori, R. F., Bettiol, W. and Athayde-Sobrinho, C. 2000. Control of damping-off in bean plant caused by *Sclerotinia sclerotiorum* by the incorporation of organic matter in the substrate. *Summa Phytopathol*. 26: 94–97.

Vicentini, C. B., Brandolini, V., Poli, T., Veronse, A. C., Guarneri, M. and Giori, P. 1990. Fungitoxicity of 6H-pyrrazole [3–4-C] [1, 2, 5] thiodiazine 2, 2-dioxides. *Pesticide Sci*. 28: 449–455.

Vieira, R. F., Pinto, C. M. F. and Mizubuti, E. S. G. 2001. Plant densities and fungicide effects on the intensity of white mould (*Sclerotinia sclerotiorum*) of dry beans. In: *Proceedings of Sclerotinia 2001. The XI International Sclerotinia Workshop* (C. S. Young and K. J. D. Hughes, eds.). York 8–12 July 2001, Central Science Laboratory, York, England, pp. 109–110.

Virtanen, A. I. and Hietala, P. K. 1958. Isolation of an anti-*Sclerotinia* factor, 7-hydroxy-4, methoxy-isoflavone from red clover. *Acta Chum. Scand*. 12: 579–580.

Vitasek, V. 1994. Resistance of winter swede rape to *Phoma lingam* and *Sclerotinia sclerotiorum. Oilseed crops. XVIth. Polish Res. Conf.* 19–20 April 1994. *Rosliny Oleiste* 15: 87–92.

Voros, J. 1969. *Coniothyrium minitans* Campbell, a new hyperparasitic fungus in Hungary. *Acta Phytopathol. Acad. Sci. Hung*. 4: 221.

Vrandecic, K., Cosic, J., Jurkovic, D. and Duvnjak, T. 2003. Weeds as an inoculum source of *Sclerotinia sclerotiorum*. Zbornik predavanj in referatov *6 Slovenskega Posvetovanje o Varstvu Rastlin, Zrece, Slovenije*, 4–6 Marec, pp. 101–106.

Vuong, T. D. and Hartman, G. L. 2003. Evaluation of soybean resistance to *Sclerotinia* stem rot using reciprocal grafting. *Plant Dis*. 87: 154–158.

Wadsworth, D. F. 1979. *Sclerotinia* blight of peanut (*Arachis hypogaea*) cultivar Comet, in Oklahoma, USA and occurrence of the sexual stage of pathogen. *Peanut Sci*. 6: 77.

Wagner, F., Kusserow, H. and Schafer, W. 2000. Cloning and targeted disruption of two polygalacturonase genes in *Penicillium olsonii. FEMS Microbiol. Lett*. 186: 293–299.

Wakefield, E. N. 1924. On the names *Sclerotinia sclerotiorum* (Lib.) Massee and *S. libertiana* Fuckel. *Phytopathology* 14: 126–127.

Waksman, G., Keon, J. P. and Turner, G. 1991. Purification and characterization of two endopolygalacturonases from *Sclerotinia sclerotiorum. Biochem. Biophys. Acta*. 1073: 43–48.

Walcz, I., Parsa, A. S., Eniody, L. and Szabo, L. G. 1985. Detection of *Sclerotinia sclerotiorum* in sunflower by enzyme linked immunosorbent assay (ELISA). *Trans. Br. Mycol. Soc*. 85: 485–488.

Walker, J. C. 1969. *Plant Pathology*, 3rd ed. McGraw-Hill, New York, 819 p.

Wallace, M. M. 1944. *Sclerotinia* disease of beans and other crops. *East Afr. Agric. J*. 9: 171.

Wallen, V. R. and Sutton, M. D. 1967. Observation on *Sclerotinia* rot of field beans in Southwestern Ontario and its effect on yield. *Can. Plant Dis. Surv*. 47: 116.

Wang, C. R., Chen, C. F., Chen, J. and Fu, J. F. 1995. *Sclerotinia ginseng*, a new species of *Sclerotinia. Acta Mycologica Sinica* 14: 187–191.

Wang, Han Zhong, Liu, GUI Hua, Zheng, Yuan Ben, Wang, Xin Fa and Yang, Qing. 2004. Breeding of the *Brassica napus* cultivar *Zhongshuang* 9 with high-resistance to *Sclerotinia sclerotiorum* and dynamics of its important defense enzyme activity. *Scientia Agric. Sinica*. 37: 23–28.

Wang, S. C. and LeTourneau, D. 1971. Carbon sources, growth, sclerotium formation and carbohydrate composition of *Sclerotinia sclerotiorum. Arch. Mikrobiol*. 80: 219–233.

Wang, S. C. and LeTourneau, D. 1972. Amino acid as nitrogen source for growth and sclerotium formation in *Sclerotinia sclerotiorum. Trans. Br. Mycol. Soc*. 59: 509–512.

Watson, A., Snudden, M., Valenzisi, J. and Ryan, K. 2002. Controlling *sclerotinia* in lettuce: part of project VG98048 lettuce IPM. *2nd Australian Lettuce Industry Conf. Paddock to Plate, Gatton*, Queensland, Australia, 5–8 May, 2002. pp. 60–62.

Watson, A. K. 1991. The classical approach with plant pathogens. In: *Microbial Control of Weeds* (D. O. TeBeest, ed.). Chapman & Hall, New York, pp. 3–23.

Watson, A. K. and Miltimore, J. R. 1975. Parasitism of the sclerotia of *Sclerotinia sclerotiorum* by *Microsphaeropsis centaureae. Can. J. Bot*. 33: 2458–2461.

Watson, A. K., Copeman, R. J. and Renney, A. J. 1974. A first record of *Sclerotinia sclerotiorum* and *Microsphaeropsis centaureae* on *Centaurea diffusa. Can. J. Bot*. 52: 2639–2640.

Weber, Z. 2002. Efficacy of biopreparate Contans WG (*Coniothyrium minitans* Campb.) in winter oilseed rape protection against *Sclerotinia sclerotiorum* (Lib.) de Bary. *Rosliny Oleiste* 23: 151–156.

Weete, J. D., Weber, D. J. and LeToureau, D. 1970. Hydrocarbons, free fatty acid and amino acids of sclerotia of *Sclerotinia sclerotiorum. Arch. Mikrobiol*. 75: 59–66.

Wegulo, S. N., Yang, X. B. and Martinson, C. A. 1998. Soybean cultivar responses to *Sclerotinia sclerotiorum* in field and controlled environment studies. *Plant Dis*. 82: 1264–1270.

Wegulo, S. N., Sun, P., Martinson, C. A. and Yang, X. B. 2000. Spread of *Sclerotinia* stem rot of soybean from area and plant sources of apothecial inoculum. *Can. J. Plant Sci*. 80: 389–402.

Weiss, A., Hipps, L. E., Blad, B. L. and Steadman, J. R. 1980a. Comparison of within canopy microclimate and white mould disease (*Sclerotinia sclerotiorum*) development in dry edible beans as influenced by canopy structure and irrigation. *Agric. Meteorol*. 22: 11–21.

Weiss, A. R., Kerr, D. and Steadman, J. R. 1980b. Temperature and moisture influence on development of white mold disease (*Sclerotinia sclerotiorum*) on great Northern beans. *Plant Dis*. 64: 757–759.

Weld, R. J., Eady, C. C. and Ridgway, H. J. 2005. *Agrobacterium* mediated transformation of *Sclerotinia sclerotiorum. J. Microbiol Meth*. (Doli: 10. 1016/j mimet. 2005.07.010).

Weld, R. J., Eady, C. C. and Ridgway, H. J. 2006. *Agrobacterium*-mediated transformation of *Sclerotinia sclerotiorum. J. Microbiol. Meth*. 65: 202–207.

Welty, R. E. and Busbice, T. H. 1978. Field tolerance in alfalfa to *Sclerotinia* crown and stem rot. *Crop Sci*. 17: 508–509.

Whetzel, H. H. 1945. A synopsis of the genera and species of the *Sclerotinia*ceae, a family of stromatic inoperculate discomycetes. *Mycologia* 37: 648–714.

Whipps, J. M. 1987. Behaviour of fungi antagonistic to *Sclerotinia sclerotiorum* on plant tissue segments. *J. General Microbiol*. 133: 1495–1501.

Whipps, J. M. 1993. Growth of the collembolan *Folsomia candida* on cultures of the mycoparasite *Coniothyrium minitans* and sclerotia of *Sclerotinia sclerotiorum. Mycol Res*. 97: 1277–1280.

Whipps, J. M. and Budge, S. P. 1993. Transmission of the mycoparasite *Coniothyrium minitans* by collembolan *Folsomia candida* (Collembola: Entomobryidae) and glasshouse sciarid *Bradsia* sp. (Diperta: Sciaridae). *Ann. Appl. Biol*. 123: 165–171.

Whipps, J. M. and Gerlagh, M. 1992. Biology of *Coniothyrium minitans* and its potential for use in disease biocontrol. *Mycol. Res*. 96: 897–907.

Whipps, J. M., Budge, S. P., Mc Clement, S. and Pink, D. A. C. 2002. A glasshouse cropping method for screening lettuce lines for resistance to *Sclerotinia sclerotiorum. Eur. J. Plant Pathol*. 108: 373–378.

Widman, L. G., Smith, O. W., Simpson, C. R. and Taber, R. A. 1992. Inheritance of resistance to *Sclerotinia minor* in selected spanisis peanut crosses. *Peanut Sci*. 19: 31–35.

Willetts, H. J. 1978. Sclerotium formation. In: *The Filamentous Fungi* (J. E. Smith and D. R. Berry, eds.). Wiley, New York, 3: 197–213.

Willetts, H. J. 1997. Morphology, development and evaluation of stromata sclerotia and macroconidia of the *Sclerotinia*ceae. *Mycol. Res*. 101: 939–952.

Willetts, H. J. and Bullock, S. 1992. Developmental biology of sclerotia. *Mycol. Res*. 96: 801–816.

Willetts, H. J. and Wong, J. A. L. 1971. Ontogenetic diversity of sclerotia of *Sclerotinia sclerotiorum* and related species. *Trans. Br. Mycol. Soc*. 57: 515–524.

Willetts, H. J. and Wong, J. A. L. 1980. The biology of *Sclerotinia sclerotiorum, S. trifoliorum* and *S. minor* with emphasis on specific nomenclature. *Bot. Rev*. 46: 101–165.

Williams, G. H. and Western, J. H. 1965a. The biology of *Sclerotinia trifoliorum* Erikss. and other species of sclerotium forming fungi. I. Apothecium formation from sclerotia. *Ann. Appl. Biol*. 56: 253–260.

Williams, G. H. and Western, J. R. 1965b. The biology of *Sclerotinia trifoliorum* Erikss. and other species of sclerotium forming fungi. II. The survival of sclerotia in soil. *Ann. Appl. Biol*. 56: 261–268.

Williams, J. R. and Stelfox, D. 1979. Dispersal of ascospores of *Sclerotinia sclerotiorum* in relation to *Sclerotinia* stem rot of rapeseed. *Plant Dis. Reptr*. 63: 395–399.

Williams, J. R. and Stelfox, D. 1980a. Occurrence of ascospores of *Sclerotinia sclerotiorum* in areas of Central Alberta. *Can. Plant Dis. Surv*. 60: 51–53.

Williams, J. R. and Stelfox, D. 1980b. Influence of farming practices in Alberta on germination and apothecium production of sclerotia of *Sclerotinia sclerotiorum. Can. J. Plant Pathol.* 2: 169–172.

Williams, M. A. J. and Spooner, B. M. 1998a. *Sclerotinia borealis,* IMI Descriptions of Fungi and Bacteria. (109) Sheet 1082, CABI Bioscience, Bakeham Lane, Egham, Surrey, TW20 9TY.

Williams, M. A. J. and Spooner, B. M. 1998b. *Sclerotinia narcissicola,* IMI Descriptions of Fungi and Bacteria. (109) Sheet 1083, CABI Bioscience, Bakeham Lane, Egham, Surrey, TW20 9TY.

Williams, M. A. J. and Spooner, B. M. 1998c. *Sclerotinia trifoliorum,* IMI Descriptions of Fungi and Bacteria. (109) Sheet 1084, CABI Bioscience, Bakeham Lane, Egham, Surrey, TW20 9TY.

Williams, R. H., Whipps, J. M. and Cooke, R. C. 1998. Role of soil mesofauna in dispersal of *Coniothyrium minitans*: transmission to sclerotia of *Sclerotinia sclerotiorum. Soil Biol. Biochem*. 30: 1929–1935.

Wilson, C. L. 1969. Use of plant pathogens in weed control. *Ann. Rev. Phytopathol*. 7: 411–434.

Wilson, C. R., Little, J. A. de, Wong, J. A. L., Schupp, P. J. and Gibson, L. J. 2005. Adjustment of soil-surface pH and comparison with conventional fungicide treatments for control of lettuce drop (*Sclerotinia minor*). *Plant Pathol*. 54: 393–400.

Wilson, R. E. 1943. Tests of eradicant sprays for use against *Sclerotinia laxa* and *Coryneum beijerinckii* in apricots and almonds. *Phytopathology* 33: 506–516.

Winter, W., Burkhard, L., Banziger, R. I., Krebs, H., Gindrat, D., Frei, P., Brandle, G., Hirner, M., Fosser, H. R., Hogger, C. and Schwartz, H. F. 1993. Rape diseases: occurrence on rape varieties, effect of fungicides and preventive control measures. *Landwirtschaft Schweiz* 16: 589–596.

Wojtaszek, P. 1997. Oxidative burst: an early plant response to pathogen infection. *Biochem. J*. 322: 681–692.

Wolcan, S., Ronco, L., Bo, E. D., Lori, G. and Alippli, H. 1996: First report of disease on lisianthus in Argentinia. *Plant Dis*. 80: 223.

Wolcan, S. M. 2004. Occurrence of crown rot of gazania and gerber caused by *Sclerotinia sclerotiorum* in Argentina. *J. Plant Pathol*. 86: 263.

Wolcan, S. M. and Grego, P. J. 2005. Stem rot of *Trachellium caeruleum* and *Craspedia globosa* caused by *Sclerotinia sclerotiorum* in Argentina. *J. Plant Pathol*. 87: 243.

Wolf, F. A. and Cromwell, R.O. 1919. Clover stem rot. *N. C. Agric. Exp. Stn. Tech. Bull*. 16: 22.

Wong, J. A. L. 1978. Green bean and white mold. *Tasmanian J. Agric*. 25: 195–201.

Wong, J. A. L. and Willetts, H. J. 1973. Electrophoretic studies of soluble proteins and enzymes of *Sclerotinia* species. *Trans. Br. Mycol. Soc*. 61: 167–178.

Wong, J. A. L. and Willetts, H. J. 1974. Polyacrylamide gel electrophoresis of enzymes during morphogenesis of sclerotia of *Sclerotinia sclerotiorum. J. Gen. Microbiol*. 81: 101–109.

Wong, J. A. L. and Willetts, H. J. 1975. A taxonomic study of *Sclerotinia sclerotiorum* and related species: mycelial interactions. *J. Gen. Microbiol*. 88: 339–344.

Wong, J. A. L. and Willetts, H. J. 1979. Cytology of *Sclerotinia sclerotiorum* and related species. *J. Gen. Microbiol*. 112: 29–34.

Woodard, K. E. and Newman, J. S. 1993. First report of *Sclerotinia minor* on Texas Bluebonnet. *Plant Dis*. 77: 318.

Woronin, M. 1888. Uber die Sclerotien Krankheit der vacciniunburer. *Mem. Akad. Imp. Sci. St. Petersburg. Ser*. 7: 36 Nr.

Wright, E. R. and Palmucci, H. E. 2003. Occurrence of stem rot of chrysanthemum caused by *Sclerotinia sclerotiorum* in Argentina. *Plant Dis*. 87: 98.

Wright, E. R., Zapata, R., Delfino, O. S. F. De., Lopez, M. V. and Serille, M. 1988. Efficacy *in vitro* of antagonists of *Sclerotinia sclerotiorum* and *Sclerotinia minor. Revista de la Fucultad de Agronimia* (*Universsidad de Buenos Aires*) 9: 109–116.

Wright, E. R., Rivera, M. C., Chiesa, G. and Morisigue, D. 2005. Occurrence of *Sclerotinia* stem rot of *Osteospermum* sp., *Felicia amelloides* and *Ranunculus asiaticus* in Argentina. *Plant Dis*. 89: 1014.

Wu, B. M. and Subbarao, K. V. 2003. Effect of irrigation and tillage on temporal and spatial dynamics of *Sclerotinia minor* sclerotia and lettuce drop incidence. *Phytopathology* 93: 1572–1580.

Wu, C. R. and Liu, H. L. 1991. Selection of rape (*Brassica napus* L) callus cultures resistant to oxalic acid. *Cruciferae Newslett*. 16–15: 80–81.

Wu, W. S. 1981. Management of sunflower *Sclerotinia* rot with chemicals. *Bot. Bull. Acad. Sinica*. 22: 75.

Wu, W. S. 1991. Control of *Sclerotinia* rot of sunflower and chrysanthemum. *Plant Prot. Bull.* [Taipei] 33: 45–55.

Wu, Y. S. and Wang, C. G. 1983. *Sclerotinia asari* Wu and Wang: a new species of *Sclerotinia*ceae. *Acta Phytopathol. Sin*. 13: 9–14.

Xie, C. J., Ruan, X. B., Cheng, J. X., Zeng, H. M., Shi, Y. P., Zhang, K. C. and Lin, D. X. 1999. Control effect of agricultural antibiotic 2–16 against *Sclerotinia sclerotiorum. Chinese J. Biol. Control* 15: 118–120.

Yakutkin, V. I. 1991a. Harmful diseases of sunflower. *Rev. Plant Pathol*. 48: 2895 (Abstr.).

Yakutkin, V. I. 1991b. Sunflower resistance to *Sclerotinia* grey rot and *Phomopsis. Zashchita Rastenii* (Moskva) 1: 41.

Yamamoto, W., Oyasu, N. and Iwasaki, A. 1956. Studies on the leaf blight disease of *Allium* spp. caused by *Botrytis* and *Botryotinia* fungi. *Sci. Reptr*. Hyogo Univ. Agric. *Agric. Biol. Ser*. 2: 17–22.

Yanar, Y., Sahin, F. and Miller, S. A. 1996. First report of stem and fruit rot of pepper caused by *Sclerotinia sclerotiorum* in Ohio. *Plant Dis*. 80: 342.

Yang, S. Z. and Li, X. J. 1990. Preliminary study on resistance to *Sclerotinia sclerotiorum* (Lib.) de Bary, in sunflower varieties. *Crop Genet. Resource* 4: 34.

Yang, X. B. and Lundeen, P. 2001. Use of herbicide induced resistance to control soybean *Sclerotinia* stem rot. In: *Proceedings of Sclerotinia 2001, the XI International Sclerotinia Workshop* (C. S. Young and K. J. D. Hughes, eds.). York 8–12 July 2001, Central Science Laboratory, York, England, pp. 169–170.

Yang, X. B., Workneh, F. and Lumsden, P. 1998. First report of sclerotium production by *Sclerotinia sclerotiorum* in soil on infected soybean seed. *Plant Dis*. 82: 264.

Young, C. S., Clarkson, J. P., Smith, J. A., Watling, M., Phelps, K. and Whipps, J. M. 2004. Environmental conditions influencing *Sclerotinia sclerotiorum* infection and disease development in lettuce. *Plant Pathol*. 53: 387–397.

Young, N. and Ashford, A. E. 1996. The effects of rind damage and regeneration on permeability of the apoplast in sclerotia of *Sclerotinia minor. New Phytol*. 134: 13–24.

Young, P. and Morris, H. E. 1927. *Sclerotinia* wilt of sunflower. *Univ. Montana, Agric. Exp. Stn. Bull*. 208: 32.

Yu, W. D., He, J. and Miao, G. F. 1995. Effective control methods of rape *Sclerotinia* rot (*Sclerotinia sclerotiorum*) in the South area of Shanxi. *Bull. Agric. Sci. Technol*. 1: 31.

Yuan, Qing Hua, Zhang, Wen Shu, Zhou, Wei Xing and Bai, Shu Juan. 2003. A preliminary study of *Sclerotinia trifoliorum* on alfalfa. *Plant Prot*. 29: 22–24.

Yuen, G. Y., Godoy, G., Steadman, J. R., Kerr, E. D. and Craig, M. L. 1991. Epiphytotic colonization of dry edible bean by bacteria antagonistic to *Sclerotinia sclerotiorum* and potential for biological control of white mould disease. *Biol. Control*. 1: 293–301.

Zakeri, Z. 1998. *Sclerotinia* rot in green house grown cucumbers. *Iran. J. Plant Pathol*. 34: 233–234.

Zandoki, E., Szodi, S. and Turoczi, G. 2005a. Mycelial compatibility of *Sclerotinia sclerotiorum* strains of different areas. *Acta Phytopath. et Entomologica Hungarica* 40: 295–301.

Zandoki, E., Szodi, S. and Turoczi, G. 2005b. Vegetative compatibility of *Sclerotinia sclerotiorum* (Lib.) de Bary strains isolated in Hungary. *Acta Phytopathologica et Entomologica Hungarica* 40: 289–294.

Zazzerini, A. and Tosi, L. 1985. Antagonistic activity of fungi isolated from sclerotia of *Sclerotinia sclerotiorum. Plant Pathol*. 34: 415–421.

Zewain, Q. K., Bahadur, P. and Sharma, P. 2004. Effect of fungicides and neem extract on mycelial growth and myceliogenic germination of *Sclerotinia sclerotiorum. Indian Phytopath*. 57: 101–103.

Zhang, Xi Lin, Zhang, Gu Feng, Zhang, Zhi Zhang, Jian Ming and Zhang, Hong Jin. 1998. Relation between percentage of rape flowering plants and optimum period for chemical control of *Sclerotinia sclerotiorum. Jiangsu J. Agric. Sci*. 14: 249–251.

Zhang, Yilan 2004. Biocontrol of *Sclerotinia* stem rot of canola by bacterial antagonists and study of biocontrol mechanism involved. M.Sc. thesis, Faculty of Postgraduate studies, University of Manitoba, Winnipeg, Manitoba, Canada, p. 137.

Zhang, Yong Hua, Gao, Hui Lan, Ma, Gui Zhen, Li, Shi Dong. 2004. Mycoparasitism of *Gliocladium roseum* 67–1 on *Sclerotinia sclerotiorum. Acta Phytopathol. Sinica* 34: 211–214.

Zhang, Yong Jie, Han, Ju Cai, Liu, Hui Ping, Gao, Jun Ming. 2004. Studies on artificial incubation method of sclerotia of *Sclerotinia sclerotiorum. Plant Prot*. 30: 74–77.

Zhao, J. and Meng, J. 2003a. Genetic analysis of loci associated with partial resistance to *Sclerotinia sclerotiorum in* rapeseed (*Brassica napus* L.). *Theor. Appl. Genet*. 106: 759–764.

Zhao, J. and Meng, J. 2003b. Detection of loci controlling seed glucosinolate content and their association with *Sclerotinia* resistance in *Brassica napus. Plant Breed*. 122: 19–23.

Zhao, J., Peltier, A. J., Meng, J., Osborn, T. C. and Grau, C. R. 2004. Evaluation of *Sclerotinia* stem rot resistance in oilseed *Brassica napus* using a petiole inoculation technique under greenhouse conditions. *Plant Dis*. 88: 1033–1039.

Zhou, Ting and Boland, G. J. 1999. Mycelial growth and production of oxalic acid by virulent and hypovirulent isolates of *Sclerotinia sclerotiorum. Can. J. Plant Pathol*. 21: 93–99.

Zhou, T. and Reeleder, R. D. 1989. Application of *Epicoccum purpurescens* spores to control white mold of snap bean. *Plant Dis*. 73: 639–642.

Zhou, T. and Reeleder, R. D. 1990. Selection of strains of *Epicoccum purpurescens* for tolerance to fungicides and improved biocontrol of *Sclerotinia sclerotiorum. Can. J. Microbiol*. 36: 754–759.

Zhou, T. and Reeleder, R. D. 1991. Colonization of bean flowers by *Epicoccum purpurascens. Phytopathology* 81: 774–778.

Zhou, T., Reeleder, R. D. and Sparace, S. A. 1991. Interaction between *Sclerotinia sclerotiorum* and *Epicoccum purpurescens. Can. J. Bot*. 69: 2503–2510.

Zhou, T., Reeleder, R. D. and Sparace, S. A. 1996. Influence of nutrients on growth of *Epicoccum nigrum. Can. J. Microbiol*. 42: 647–654.

Zhu, J. L. and Chen, X. R. 1999. Screening of resistance in cucumber varieties to *Sclerotinia sclerotiorum* at seedling stage. *Plant Prot*. 25: 10–12.

Zhu, J. L. and Chen, X. R. 2000. Screening of resistance to *Sclerotinia sclerotiorum* in cucumber varieties. *Plant Prot*. 26: 10–12.

Ziman, L. 1997. Microanatomy of the fungus *Sclerotinia sclerotiorum. Biol. Bratislava*. 52: 389–394.

Ziman, L., Paul, V. H. (ed.), Kohr, K. (ed.), Thomas, J. (ed.) and Dupprich, P. D. 1998. Resistance of some sunflower hybrids to *Sclerotinia* wilt. Integrated control in oilseed crops. In: *Proceedings on meeting held at Poznan*, Poland, 10–12, April 1997. *Bull. OILB-CROP* 21: 137–140.

Zimmer, D. E. and Hoes, J. A. 1978. *Diseases in Sunflowers Science and Technology* (J. F. Carter, eds.). American Society of Agronomy/Crop Science Society of America/Soil Science Society of America, Madison, WI, Chap. 7.1.

Zimmer, R. C. and Campbell, C. 1990. First report of *Sclerotinia sclerotiorum* on *Lathyrus sativus. Can. Plant Dis. Surv*. 70: 17–18.

Zoina, A., Raio, A., Lorito, M. and Noviello, C. 1990. *In vitro* antagonism of *Sepedonium chrysospermum* (Bull.) Link. Fr. to *Sclerotinia sclerotiorum* (Lib.) de Bary and *Rhizoctonia solani*. Kuhn. *Annali della Facolta di Scienze Agrarie della universita degli studi di Napoli, Portici*. 24: 63–75.

Zuppini, A., Navazio, L., Sella, L., Castiglioni, C., Favaron, F. and Mariani, P. 2005. An endopolygalacturonase from *Sclerotinia sclerotiorum* induces calcium mediated signaling and programmed cell death in soybean cells. *Mol. Plant Microbe Interact*. 18: 849–855.

Subject Index